S02: Understanding ANSI/ASB Best Practice Recommendation 037: Guidelines for Opinions and Testimony in Forensic Toxicology

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In January 2019, the American National Standards Institute (ANSI) published a best practice recommendation for offering opinions and testimony in the field of forensic toxicology. While the document was drafted by the Toxicology Subcommittee of the Organization of Scientific Area Committees (OSAC), the AAFS Standards Board (ASB), an ANSI-accredited Standards Development Organization, took responsibility for reviewing the document, circulating it to the general public, and adjudicating the comments received on the document. Following multiple circulations of the document to solicit public comments, the final version of the document was accepted by ANSI.

The document is intended for the subdisciplines of human performance toxicology, postmortem forensic toxicology, non-regulated employment drug testing, court-ordered toxicology, and general forensic toxicology. It is divided into sections to specifically address what is generally considered to be "appropriate" opinions and testimony by the field, as opposed to opinions and testimonies that are generally "inappropriate".

Attendees will learn the history of the development of the document and how it relates to other ANSI/ASB published and OSAC draft documents. The key recommendations of the document will be reviewed and, where appropriate, examples will be provided. The goal is to help the attendee understand the document and so that they feel comfortable using it in their daily practice.

Free copies of *ANSI/ASB Best Practice Recommendation 037: Guidelines for Opinions and Testimony in Forensic Toxicology* can be obtained at www.asbstandardsboard.org.

PLATFORM

S03: Impaired Driving Toxicology Data Collection

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Background/Introduction: Impaired driving is a major concern in the United States and around the world. There is growing attention being focused on drug-impaired driving. However, there are insufficient data to understand the true magnitude and scope of the problem. The prevalence of drugged driving is underrepresented because many laboratories do not test for drugs when the blood alcohol concentration is above a certain predetermined level. The same core set of drugs are not tested in all cases, so data generated from different laboratories cannot be combined into a meaningful data set. Limitations with data collection systems, and variability among states, further impacts the ability to obtain quality information. These issues make it difficult to develop data driven public policies to reduce the negative impacts of impaired driving. The increase in states legalizing marijuana has exacerbated the need for reliable data to assess impacts and direct policies.

Objectives: To inform the forensic toxicology community about the limitations of the current impaired driving data systems and encourage them to collaborate to improve the quality and relevance of the toxicology data available.

Methods: The National Highway Traffic Safety Administration (NHTSA) is working to address limitations with the drug-impaired driving data in its Fatality Analysis Reporting System (FARS). FARS data provide a crucial metric for traffic safety professionals and policy makers. Current limitations make it impossible to use FARS data to determine the scope and magnitude of the drugged-driving problem or make inferences about impairment, crash causation, or compare drug and alcohol related fatal crashes.[1] Similar issues with drug-impaired driving data exist in other data systems across the country. This presentation will highlight the limitations with current drug-impaired driving data and the need for better data, possible improvements in toxicological testing, such as conducting Tier 1 testing[2] for all impaired driving cases, and possible improvements in data systems and data dissemination.

Results and Conclusion/Discussion: Creating consistency in the scope and sensitivity of toxicology testing in impaired driving investigations and expanding the capability of data systems to include full toxicology results, will greatly enhance the amount of quality data available. Improving the integration of toxicology testing into new/existing data systems will allow all stakeholders to have comprehensive meaningful information on the scope of the impaired driving problem and allow for the development of data driven policies to combat that problem.

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2 Logan, B., D'Orazio, A., Mohr, A., Limoges, J., Miles, A., Scarneo, C., Kerrigan, S., Liddicoat, L., Scott, K., and Huestis, M. Recommendations for Toxicological Investigation of Drug-Impaired Driving and Motor Vehicle Fatalities – 2017 Update. Journal of Analytical Toxicology. 42,2, March 2018, pages 63-68.

S04: In Vitro Transformation of CBD to THC in Forensic Toxicology Hair Testing – Artifact or Fiction?

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Background/Introduction: The prevalence of cannabidiol (CBD)-infused products on the market has invaded most aspects of everyday life, including health and beauty, food consumables, and even dog treats. While similar in structure to tetrahydrocannabinol (THC), CBD differs widely in its properties. Most notably, it does not bind to CB1 receptors and lacks the characteristic psychoactive effects ascribed to THC. Though CBD products can be sold in CBD-only forms, more often than not, CBD marketed products also contain small quantities of THC. To add further confusion, recent literature provides conflicting reports on whether CBD can be converted to THC in acidic environments, such as those used during a forensic toxicology extraction process. This leaves the toxicologist to interpret whether positive THC results could be consistent with THC exposure, CBD consumption, or present as an artifact of the analysis method used.

Objectives: In 2016, a study by Merrick et al. noted that when exposed to an acidic environment in vitro, CBD can be transformed to THC and other cannabinoid compounds. We present here an evaluation of CBD to THC transformation as it relates to a THC hair analysis method using solid phase extraction (SPE) and gas chromatography mass spectrometry (GC-MS), a technique routinely used by our laboratory.

Methods: Drug free hair specimens were fortified with CBD solution to reach final concentrations of 50, 500, 2500, and 5000 pg/mg. The specimens were digested with strong base (1N NaOH, 1 hour). After cooling, the supernatants were brought to pH 4.5 with acetic acid and 0.1M sodium acetate buffer. The SPE columns were conditioned with methanol, deionized water, and 0.1N HCl. The supernatants were added, and each column was rinsed with deionized water and a mixture of 0.1N HCl and acetonitrile. The final elution was achieved with hexane which was subsequently evaporated to dryness. The dry residues were derivatized with BSTFA and 1% TMCS and analyzed by GC-MS in an electron ionization mode (EI). The cutoff for THC was 10 pg/mg.

Results: CBD hair concentrations of 50 pg/mg and 500 pg/mg did not produce THC levels exceeding the cutoff value of 10 pg/mg. However, the results of analysis of 2500 pg/mg and 5000 pg/mg CBD hair preparations indicate that, in the presence of acidic conditions applied throughout the analytical process, CBD may convert to THC to a small extent not to exceed 2% of the relative CBD concentration.

Conclusion/Discussion: In vitro transformation of CBD to THC using common toxicological analysis techniques has been demonstrated. Each laboratory conducting hair analysis for THC should determine the potential extent of THC formation during the analytical process applied.

PLATFORM

S05: Fatalities Involving the Synthetic Cannabinoid, 5-Fluoro-ADB: Forensic Pathology and Toxicology Implications

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Background/Introduction: 5-Fluoro-ADB has become an increasingly popular drug of abuse as evidenced by the magnitude of confiscations reported in Europe and the increase in the number of acute intoxications and fatalities reported worldwide. The psychological and behavioral effects of 5-Fluoro-ADB share similarities with cannabis, including relaxation, euphoria, lethargy, confusion, anxiety, fear, distorted perception of time, depersonalization, hallucinations, paranoia, dry mouth, bloodshot eyes, tachycardia, nausea, vomiting, and impaired motor function. Aggressive behavior, violence and psychotic episodes have also been reported, suggesting that the effects are much more severe when compared to cannabis. Although reports describing acute and fatal intoxications involving 5-Fluoro-ADB are becoming more prevalent, very limited information has been described regarding the terminal event, autopsy findings, parent and metabolite drug concentrations in multiple drug sites, and cause of death.

Objectives: The objective is to present forty-three fatalities involving the potent synthetic cannabinoid, 5-Fluoro-ADB. Correlation between terminal event, autopsy findings, cause of death, and concentration of 5-Fluoro-ADB and its ester hydrolysis metabolite, 5-Fluoro-ADB Metabolite, in multiple blood specimens will be discussed.

Methods: Bond Elute Plexa PCX solid phase extraction columns were used to isolate the target analytes from postmortem blood specimens. Extracts were submitted to an Agilent 1290 HPLC system coupled to an Agilent 6460 electrospray triple quadrupole mass spectrometry for analysis. Separation was achieved using an Agilent PFP Poroshell 120 (4uMx3x50mm) analytical column with an aqueous mobile phase of 5mM ammonium formate with 0.1% formic acid in LC-MS grade water, and an organic mobile phase of LC-MS grade acetonitrile with 0.1% formic acid. The linear range of the method is 0.010 – 10 ng/mL for 5-Fluoro-ADB and 10 – 500 ng/mL for 5-Fluoro-ADB Metabolite, with a limit of detection of 0.010 ng/mL and 0.50 ng/mL, respectively.

Results: Central blood concentrations ranged from 0.010 to 2.2 ng/mL (average: 0.34 ng/mL) for 5-Fluoro-ADB and 2.0 to 166 ng/mL (average: 41 ng/mL) for 5-Fluoro-ADB Metabolite. Peripheral blood concentrations ranged from 0.010 to 0.77 ng/mL (average: 0.15 ng/mL) and 2.0 to 110 ng/mL (average: 21 ng/mL) for 5-Fluoro-ADB and 5-Fluoro-ADB Metabolite, respectively. 5-Fluoro-ADB central to peripheral blood concertation ratios (C/P) greater than 1 was reported for 58% of the cases, whereas 71% of the cases resulted in 5-Fluoro-ADB Metabolite C/P greater than 1. Non-specific findings at autopsy included pulmonary congestion and edema and aspirated gastric contents; cardiac weights measured for the decedents were between 280 – 710 grams, with lung weights varying between 270 – 1460 grams. The volume of gastric contents recorded for 78% of the cases was greater than 100 mL.

Conclusion/Discussion: The low concentrations calculated confirm the necessity for sensitive analytical techniques to identify 5-Fluoro-ADB in postmortem blood specimens. 5-Fluoro-ADB Metabolite is present in much greater concentrations in blood than 5-Fluoro-ADB, suggesting its use as a marker for synthetic cannabinoid abuse. Furthermore, the usefulness of screening central blood is evident based on its increased concentration, possibly due to postmortem redistribution, when compared to peripheral blood concentrations. Combining the toxicological and pathological findings, it can be hypothesized that individuals with cardiomegaly may be more susceptible to the adverse effects of 5-Fluoro-ADB. In addition, the physical demand on cardiac output in the post-prandial period can precipitate a dysrhythmia and sudden death.

Keywords:

5-FLUORO-ADB, SYNTHETIC CANNABINOIDS, POSTMORTEM REDISTRIBUTION

S06: Interpretation Issues in a Death Involving Gliclazide, an Oral Hypoglycemic Drug

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Background/Introduction: Diabetes mellitus type 2 (non-insulin dependent diabetes) is a chronic metabolic syndrome. Major symptoms include hyperglycemia and aberrant metabolism of proteins, fats and carbohydrates. Control of blood glucose concentrations is possible by appropriate medication. Several classes of anti-diabetics are available, including sulphonylureas. Treatment with sulphonylureas is potentially life threatening by hypoglycemia. Gliclazide, a member of this class, is on the WHO list of essential medicines. Although its used by millions of patients around the world, little postmortem data is available for gliclazide.

Objectives: An unexpected death of a 46 year-old woman presenting a type II diabetes was observed and the Prosecutor requested an autopsy followed by toxicological investigations. Autopsy findings (labial ecchymosis, multi viscera congestion, asphyxia syndrome, moderate cerebral edema) were in accordance with a possible hypoglycemia death. Toxicological analyses, performed 6 weeks after the death (while specimens were stored at + 4°C), revealed the presence of gliclazide in femoral blood at 2.2 mg/L, which is in the range of published therapeutic concentrations. During a meeting with the pathologist, 3 possible explanations were discussed: 1. unknown cause of death (other than a toxic death), 2. death due to gliclazide which could have been degraded due to chemical instability (at the time of death the blood concentration could have been much higher), and 3. blood concentration was enough to produce fatal hypoglycemia in a non-observant patient. The objectives of this presentation are to discuss, based on literature survey and additional tests, these possible scenarios.

Methods: During autopsy, cardiac blood, femoral blood, vitreous humor, gastric content and hair (6 cm, dark) were collected. Gliclazide, identified during a comprehensive screening, was tested in the various specimens by LC-MS/MS after acid extraction. To document chemical stability, 20 mL of blood were spiked with gliclazide for a final concentration at 10 mg/L, then divided into 1.2 mL vials, kept at + 4 °c and – 20 °C. Specimens were tested over 3 months.

Results: Blood alcohol was negative. Toxicological screening detected gliclazide, which was then quantified using a MRM method. The following concentrations were measured: femoral blood (2199 ng/mL), cardiac blood (1949 ng/mL), vitreous humor (36 ng/mL), and gastric content (< 10 ng/mL). Hair tested also positive in the 3 x 2 cm segments, at 7, 8 and 3 pg/mg. No other drug, including pharmaceuticals, drugs of abuse and NPS was identified. After 3 months, a final loss of about 35 % (at – 20 °C) and 70 % (at + 4 °C) of gliclazide was observed, indicating chemical instability.

Conclusion/Discussion: The major issue in this case is the therapeutic concentration of gliclazide detected in the femoral blood of the victim. Although a fatal gliclazide concentration has never been described in the literature, the question was to evaluate possible death in such circumstances. Testing for gliclazide stability in whole blood over a period that matches the delay of the toxicological analyses did not demonstrate massive instability (loss of about 30 % after 6 weeks storage at + 4 °C). On the opposite, segmental hair results, with low concentrations over 6 months, were highly indicative of non-compliant use of the medicine. In subjects under gliclazide therapy, hair concentrations (n=6) were in the range 550-1200 pg/mg, much higher than what was measured in the hair of the victim. Although not studied nor reported, one can anticipate good gliclazide stability in hair, as it is the case for all other drugs when the specimen is stored dry at room temperature. It was therefore concluded by the pathologist that the cause of death of the subject was an inappropriate use of gliclazide, a drug that could be responsible of a fatal hypoglycemia, even at normal therapeutic concentration.

PLATFORM

S07: Δ-9-Tetrahydrocannabinol Distribution in Central Blood, Peripheral Blood, and Brain Tissue

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Background/Introduction: Once ingested, Δ-9-tetrahydrocannabinol (THC) quickly moves out of the blood and into fatty tissues, including the brain. As a result, concentrations in the blood may not represent concentrations in the brain, making correlation to impairment difficult, at best. Postmortem redistribution may also complicate interpretation of the involvement of the drug in the death. Postmortem blood and tissue samples were analyzed and evaluated for individuals who died in traumatic collisions while driving a motor vehicle and compared with individuals who died of other causes.

Objectives: The objective of this research was to quantitate THC in brain tissue and compare concentrations to corresponding central blood and peripheral blood samples.

Methods: Forty-three THC positive central blood samples were selected. Twenty-one of the deceased individuals were drivers in fatal automobile collisions, while the other 22 died of other causes (natural, homicide, suicide, etc.). When available, central cardiac blood, peripheral blood, and brain tissue were analyzed.

Blood was sampled at 150 μL and homogenized brain tissue (2x) at 0.6 g. The sample was mixed with 0.1% formic acid and deuterated internal standard (THC-d3) then added to an Isolute SLE+ column. The extracts were eluted with 70:30 hexane:ethyl acetate. Samples were dried with heated air (50 - 70 °C) and reconstituted in mobile phase.

Separation occurred on a Waters Aquity UPLC with HSS T3 column using aqueous and organic phases of 100% water and acetonitrile, each with 0.1% formic acid. The LC method consisted of a 5-minute gradient. A Waters XeVo-TQS collected MRM data in ESI+ mode with two ion transitions. The quantitative range is 1-100 ng/mL for THC on a quadratic curve, with the administrative LOD set at the LOQ. This method was previously validated with SWGTOX validation standards.

Ratios for THC concentrations were calculated for all blood and brain tissue combinations.

Results: Among driver cases, eight of 21 peripheral bloods and seven of 14 brain samples tested had concentrations greater than the central blood concentrations. All brain samples tested were positive for THC. In the non-driver cases, 13 of 21 peripheral bloods and eight of 22 brain samples tested had concentrations greater than the central blood concentrations. In some cases, brain tissue did not contain quantifiable amounts of THC when it was detected in central blood. The table below summarizes the observed ratios between all sample types.

THC Concentration Ratios				
Central/Peripheral Blood; **Central Blood/Brain Tissue;** <u>Peripheral Blood/Brain Tissue</u>				
	n=	**Mean**	**Median**	**Range**
Drivers	14;	1.33;	0.91;	0.32 to 5.70;
	13;	**1.35;**	**0.96;**	**0.15 to 3.07;**
	<u>13</u>	<u>1.06</u>	<u>1.05</u>	<u>0.06 to 3.62</u>
Non-Drivers	17;	0.94;	0.67;	0.12 to 2.95;
	17;	**2.16;**	**0.96;**	**0.28 to 9.28;**
	<u>14</u>	<u>2.17</u>	<u>0.76</u>	<u>0.24 to 7.81</u>
Total	31;	1.12;	0.81;	0.12 to 5.70;
	30;	**1.80;**	**0.96;**	**0.15 to 9.28;**
	<u>27</u>	<u>1.63</u>	<u>0.79</u>	<u>0.06 to 7.81</u>

Conclusion/Discussion: Interpretation of postmortem THC concentrations is difficult due to the nature of the drug. Variations in sample collection at autopsy may also influence postmortem concentration changes. In this study, postmortem blood and tissue samples were analyzed for THC to determine if any patterns in distribution were observed when comparing concentrations. Stronger conclusions could not be drawn due to the lack of information regarding impairment and the historical cannabis consumption for the diseased. There were also limitations in the number of cases with all three sample types available for comparison. Among the driver group, all tested brain tissue was positive for THC, indicating a possibility these individuals were affected by THC at the time of the fatal collision.

PLATFORM

S08: Distribution of Synthetic Opioids in Postmortem Blood, Vitreous Humor and Brain

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Background/Introduction: In the US, the use of synthetic opioids has become an increasing health issue with thousands of overdose deaths observed since 2013. With the high mortality rate associated with these substances, postmortem analyses and interpretation of synthetic opioids has become extremely important. However, due to the novelty of these compounds, the available data are limited and provides challenges to toxicologists.

Objectives: (1) Develop and validate analytical methods for the determination of fifteen synthetic opioids (3-methylfentanyl, 4-ANPP, 4-methoxybutyrylfentanyl, acetylfentanyl, butyrylfentanyl, carfentanil, fentanyl, furanylfentanyl, isobutyrylfentanyl, MT-45, norfentanyl, p-fluorobutyrylfentanyl, U-47700, valerylfentanyl and W-18) in vitreous humor and in brain tissue; (2) Investigate the postmortem distribution and detectability of synthetic opioids within blood, vitreous humor and brain samples.

Methods: 0.5-mL vitreous humor and 3g of brain tissue homogenized in water (diluted 1:3, w/w) were extracted by mixed-mode cation exchange-reversed phase solid phase extraction. Extracts were analyzed by LC-MSMS. The internal standards employed were fentanyl-d_5 and norfentanyl-d_5. Chromatographic separation was performed by reversed-phase (Agilent Poroshell 120 EC18, 2.1 x 100mm, 2.7µm) with 0.1% formic acid in water and in acetonitrile as mobile phases in gradient mode, with a total run time of 19min. Data were acquired with ESI+ in dynamic multiple reaction mode (dMRM), monitoring two transitions per compound. The methods were validated following SWGTOX guidelines. Fifty-eight authentic case samples from the New York City Office of the Chief Medical Examiner (NYC-OCME) were analyzed. These cases were positive for at least one of the analyzed opioids, and all three matrices were available. The blood sample sources included femoral (n=42), iliac (n=2), subclavian (n=3) and cardiac (n=11).

Results: Vitreous humor was cross-validated with a previously validated blood method. All quantitative analytes had a limit of quantification of 0.1ng/mL. Quantitative analytes had acceptable bias (-5.8 - 15.9%) and precision (CV, 0.6-12.4%) at all three levels of QCs (0.5, 8, and 80ng/mL, n=15). Significant matrix effects were exhibited for MT-45, p-fluorobutyrylfentanyl, valerylfentanyl, and W-18 (up to -60.4%, CV<20%, n=10). All analytes demonstrated extraction efficiencies 65.6-84.1%, except for norfentanyl and norfentanyl-d_5 which were 29.5-31.7%. The brain extraction method had a linear range of 0.1-100ng/g. Quantitative analytes had acceptable bias (-10.1 - 11%) and precision (0.6-14.3%) for all levels of QC (0.5, 8, and 80ng/g, n=15). The majority of analytes had matrix effects <20%. Most analytes had extraction efficiencies of 50.1-80%, except for 4-methoxybutyrylfentanyl, butyrylfentanyl, furanylfentanyl, MT-45, and norfentanyl that ranged from 21.1-43.4%. No long-term stability studies were performed.

Six of the fifteen analytes (4-ANPP, acetylfentanyl, fentanyl, furanylfentanyl, norfentanyl, U-47700) were detected in the authentic cases. Concentrations were within the 0.1 to 100ng/mL or ng/g across all three matrices, with only concentrations of acetylfentanyl and U-47700 exceeding the upper limit. The highest concentrations were observed in brain (except norfentanyl), followed by blood and vitreous humor. Most analytes were detected in all three matrices in a given case (from 96% of cases for fentanyl to 39% for ANPP). 4-ANPP had the highest percentage of cases in which it was only detected in brain (24%), as well as the highest percentage of cases in which the analyte was only detected in blood and brain (22%).

Conclusion/Discussion: We developed and validated two sensitive and specific methods for the detection of fifteen synthetic opioids in vitreous humor and brain samples. The synthetic opioids in this study displayed a higher affinity for brain tissue when compared to blood and vitreous humor as presented by the larger concentrations detected in brain, except for norfentanyl. Brain tissue and vitreous humor were demonstrated to be viable alternatives in detecting the presence of synthetic opioids in place of blood.

PLATFORM

S09: Preanalytical Artifacts and Postmortem Considerations of Gabapentin

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Introduction: Gabapentin (Neurontin) is a widely prescribed drug, approved for use as an anticonvulsant for epileptic seizures and for management of post-herpetic neuralgia, though frequently prescribed for any number of off-label purposes. While gabapentin is structurally similar to the neurotransmitter GABA, it does not bind to or activate GABA receptors, and its mechanism of action remains relatively unknown.

Despite not having typical CNS depressant effects, gabapentin is widely used and abused in conjunction with illicit opioids. Data from 2015 shows gabapentin is present in 26% of North Carolina drug overdose deaths, with 96% of those cases including an opiate or opioid. Gabapentin use continues to grow, and since 2015 North Carolina has seen an 18% increase in gabapentin-positive cases.

More cases however, lead to more potential problems. With a volume of distribution near 1, gabapentin does not exhibit classical postmortem redistribution, but is not exempt from other anomalies and preanalytical artifacts. At NC OCME the toxicology lab screens for gabapentin in a central blood specimen and confirms on a peripheral blood specimen. Most of the time the screen and confirmation results closely match, however there are times where the peripheral is much greater. Since gabapentin is eliminated intact by renal excretion, the possibility of diffusion from the bladder into the iliac vein exists and could significantly increase gabapentin concentrations after death. These discrepancies, if left unchecked, could result in erroneous means and manner of death.

Objectives: The goal of the presentation is to provide examples of disparate central-to-peripheral gabapentin concentrations and how to interpret that information in the setting of a postmortem medicolegal case.

Methods: Gabapentin is screened by a validated multi-analyte targeted assay employing a high-resolution, accurate mass Thermo Orbitrap LC-MS/MS with a cut-off for confirmation of 1.0 mg/L. Confirmation and quantitation is achieved by a validated LC-MS/MS method in blood using 0.1 mL of specimen and using gabapentin-d_{10} as an internal standard. Positive electrospray ionization on a Thermo TSQ triple quadrupole LC-MS/MS monitors two transitions for each analyte with identification criteria based upon retention times and ion ratios. A whole blood linear calibration curve of 1.0 – 50 mg/L, as well as matrix matched controls is included with each batch of specimens. Our internal database was searched for cases where the gabapentin concentration of the peripheral specimen greatly exceeds the central.

Results: A few select cases are presented below. Also, to be presented are supporting instrumental information, additional case studies, and considerations for postmortem interpretation.

Conclusion: Preanalytical artifacts arising from diffusion from the bladder to the iliac vein could result in elevated gabapentin concentrations. If left unnoticed, these elevated concentrations could lead to erroneous means and manners of death. If screening on a central specimen and quantitating on a peripheral specimen it is important to compare the two concentrations, particularly when an iliac specimen is used. Great care must also be taken when a peripheral specimen is used for both screening and confirmation, particularly if gabapentin is to be invoked in the means and manner of death.

Keywords: Gabapentin, postmortem toxicology, preanalytical artifacts

S10: Post-mortem Fentanyl and Fentanyl Analog Toxicological Findings from the CDC's State Unintentional Drug Overdose Reporting System (SUDORS)

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Background/Introduction: There were 70,237 drug overdose deaths in 2017, with more than two-thirds involving opioids. The current opioid overdose epidemic has been characterized by three waves: deaths involving prescription opioids (1990s), followed by increases in deaths involving heroin (starting in 2010), and most recently deaths involving fentanyl (starting in 2013). The fentanyl wave of the epidemic has predominantly involved illicitly manufactured fentanyl, but has also included deaths attributable to fentanyl analogs, in combination with fentanyl, with each other, or alone.

Objectives: To present trends in drug overdose deaths involving fentanyl and fentanyl analogs among jurisdictions funded by CDC's Enhanced State Opioid Overdose Surveillance (ESOOS) program.

Methods: ESOOS currently funds 32 states and Washington, DC to report data on nonfatal and fatal opioid overdoses. Data on fatal opioid overdoses that were unintentional or of undetermined intent are collected within the State Unintentional Drug Overdose Reporting System (SUDORS) and reported to CDC biannually with a lag of 8–13 months after the date of death. Funded jurisdictions abstract data from death certificates and medical examiner/coroner reports, including complete postmortem toxicology test results. Data on opioid overdose deaths that occurred during July 2016–June 2018 were analyzed to determine the number of unintentional or undetermined intent overdose fatalities that involved fentanyl and fentanyl analogs.

Results: There were 40,243 opioid overdose deaths reported by 28 jurisdictions during July 2016–June 2018, with the number of reporting jurisdictions varying over time. Across all jurisdictions and periods, fentanyl was detected in 25,093 (62.4%) deaths and ≥1 fentanyl analog was detected in 8,316 (20.7%) deaths; either fentanyl, ≥1 fentanyl analog, or both, were detected in 28,027 (69.6%) deaths. All 28 states had ≥1 death each with fentanyl and any fentanyl analog detected. The most commonly detected fentanyl analogs were acetylfentanyl (n=3,089, 7.7%, 27 states); carfentanil (n=2,205, 5.5%, 19 states); cyclopropylfentanyl (1,062, 2.6%, 22 states); a combined group of "fluorofentanyls" (because of identification issues, this includes fluorobutyrylfentanyl, 4/para-fluorobutyrylfentanyl, fluoroisobutyrylfentanyl, and 4/para-fluoroisobutyrylfentanyl) (n=1,023, 2.5%, 22 states); and furanylfentanyl (n=784, 2.0%, 22 states). Fourteen additional fentanyl analogs were detected in ≥1 death. During July 2016–June 2018, there were sequential peaks in deaths with carfentanil, cyclopropylfentanyl, and furanylfentanyl detected, at 246, 140, and 113 deaths, respectively, followed by drop-offs to fewer than 50 deaths each by June 2018. There was an initial peak (113 deaths) and drop-off in detection of "fluorofentanyls;" however, there was a secondary peak with 94 deaths in May 2018. Acetylfentanyl was detected in increasingly higher numbers of deaths over time, up to a high of 322 deaths in June 2018.

Conclusion/Discussion: Fentanyl and/or a fentanyl analog were detected in over two-thirds of opioid overdose deaths reported to SUDORS during July 2016–June 2018. Most of the detected analogs followed a pattern of increasing detection over time to a peak, followed by a drop-off to a few deaths by June 2018. This decrease coincided with the temporary scheduling by DEA of all fentanyl-related substances in February 2018. Opposing this trend, the category of "fluorofentanyl" appeared to have a secondary peak, and acetylfentanyl was detected in increasing numbers of deaths over time through June 2018. This supports some existing evidence of acetylfentanyl as a potential contaminant in the production of illicitly-manufactured fentanyl, in addition to being a distinct analog. SUDORS represents a unique data source to examine complete postmortem toxicology test results of opioid overdose deaths (not limited to those substances determined to have caused the death). Continuing to monitor trends in the detection of fentanyl, fentanyl analogs, and other new psychoactive substances over time can help elucidate drug market trends and identify opportunities to prevent overdose deaths.

PLATFORM

S11: The Detection of Methyl Ether in Four Postmortem Cases and Two DUID Law Enforcement Cases

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Background/Introduction: Methyl ether, also referred to as dimethyl ether, is a flammable compound with a boiling point of -11.2°F (-24°C). It is used as a refrigerant, an aerosol propellant, and as an alternative fuel. It is also used in the synthesis of other compounds, such as dimethyl sulfate and trimethyloxonium tetrafluoroborate. The authors are aware of only one postmortem report involving inhalation of methyl ether. In that case, the substance was detected in the brain of a decomposed body. Very little is known about the concentration of methyl ether in tissues and fluids in cases in which the chemical was implicated in the death.

Objectives: The objectives of this study were to determine the concentration of methyl ether in four postmortem cases where volatile compounds were thought to play a role in the death; methyl ether concentrations were also studied in two cases of DUID where methyl ether was detected along with other drugs.

Methods: A screen for volatile substances was requested in four postmortem and two DUID cases. Blood (0.5 mL) was screened for volatile compounds by dual column head space gas chromatography and subsequently identified by gas chromatography/mass spectrometry. Methyl ether was quantified by use of a 6-point calibration curve prepared in a blood matrix. Calibrators ranged from 940 ng/mL to 47,000 ng/mL. The internal standard was 1,4-dioxane. Linear regression with a second order curve with 1/x weighting was used and typically yielded r^2 values greater than 0.999. A high and a low control were assayed with each batch. Specimens were analyzed with no dilution and with a 1:9 dilution. CV's for the high and low control are <10%. Briefly, the method is as follows. Blood, 0.5 mL, and internal standard was added to a 20 mL headspace vial and immediately sealed. Calibrators and controls were spiked with appropriate amounts of dimethyl ether. The vials were incubated at 70°C for 20 minutes then sampled and injected. The split ratio was 15:1. The column was a 30 meter X 0.32 mm ID WAX column with a film thickness of 0.50 µm. The oven was immediately programmed from 40°C to 60°C at 4°/min. A FID was used as the detector.

Results: The four postmortem cases had methyl ether blood concentrations that ranged from 200,000 ng/mL to 770,000 ng/mL (mean 488,000 ng/mL, median 490,000 ng/mL) as compared to the DUID law enforcement cases where concentrations of 3,100 and 19,000 ng/mL were detected in blood. Three of the four postmortem cases had no other significant findings while the other case had 1,1 difluoroethane (DFE) present at a concentration of 180,000 ng/mL along with 39 ng/mL of 7-aminoclonazepam, 1.0 ng/mL of Delta-9-THC, and 10 ng/mL of buprenorphine. Significant findings in addition to methyl ether were present in the two DUID cases. One case had 1,700 ng/mL of DFE and 0.55 ng/mL of Delta-9-THC in addition to 3,100 ng/mL of methyl ether. The second law enforcement case had findings of 19,000 ng/mL of methyl ether, 18 ng/mL of Delta-9-THC, 9.7 ng/mL of 11-hydroxy Delta-9-THC, and 10 ng/mL of bupropion.

Conclusion/Discussion: In the cases presented in this study, the concentrations of methyl ether in postmortem specimens were significantly higher than those detected in the law enforcement cases. However, there is always a time delay in collection of specimens in DUID cases, which may account for the lower concentrations. Interpretation of the methyl ether concentration in the DUID cases is also complicated by the presence of significant findings for other impairing substances. Unfortunately, no information was submitted concerning the observed behavior of the DUID subjects. The presence of methyl ether can be detected in routine screening for volatile compounds. In addition, laboratories should be cognizant of an early eluting peak in routine screens for ethanol, as this peak was subsequently identified as methyl ether in our postmortem cases.

PLATFORM

S12: An ISOlated Homicide

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Background/Introduction: Isopropanol is a simple alcohol commonly used as an antiseptic and household disinfectant. Persons suffering from alcohol addiction or those with suicidal intentions may consume it as an ethanol substitute due to its ease of access. It acts as a central nervous system depressant, likely affecting the brain stem, leading to respiratory depression and circulatory collapse. Isopropanol is rapidly absorbed, widely distributed, and metabolized to acetone by alcohol dehydrogenase. It is roughly twice as toxic as ethanol though deaths are rare, possibly owing to proper emergency care. In the case presented here, a 48 year old male, who was displaying increasingly bizarre behavior in the weeks leading up to the incident, forced his 82 year old female landlord to drink rubbing alcohol. He proceeded to set her on fire because he believed she was possessed by the Devil. Her body was recovered from the bedroom in her home by arson investigators with burns covering 100% of her body. The autopsy revealed that the stomach contained 14 red-orange tablets presumptively determined to be nifedipine through drug chemistry analysis as well as foam in the respiratory tract with congested lungs and aspirated gastric contents. No evidence of strangulation or soot in the airway was present. Routine toxicology testing revealed the presence of ethanol, isopropanol and acetone and a sub-therapeutic level of nifedipine. The postmortem carboxyhemoglobin blood saturation percentage was below clinical significance.

Objective: The objective of this case study was to quantify the amount of isopropanol, ethanol and acetone in the heart blood and urine of an individual suspected to have been compelled to consume rubbing alcohol. Due to the condition of the body, femoral blood and vitreous humor was not available for testing.

Method: Samples were analyzed using dual column headspace gas chromatography with flame ionization detection (HSGC-FID). 100 µL of sample was diluted with 700 µL 0.02 % n-propanol in a 20 mL headspace vial. Vials were heated to 65°C and agitated for 2 minutes prior to injection on an Agilent 7890A GC using a gas tight syringe heated to 70°C. The columns used were DB-ALC1 and DB-ALC2 with a run time of 4.5 minutes at 40°C isothermal. Confirmations of volatile positive samples were performed on a second day.

Results: Blood and urine samples collected at autopsy were each assayed and the following volatile concentrations determined:

Sample	Ethanol (g/100 mL)	Isopropanol (g/100 mL)	Acetone (g/100 mL)
Heart Blood	0.046 ± 0.004	0.175 ± 0.010	< 0.025
Urine	ND	< 0.025	ND

The ethanol was likely a component of the rubbing alcohol, and the acetone a metabolite of isopropanol. No vitreous fluid was available to ascertain glucose levels in order to exclude diabetic ketoacidosis as a source of acetone due to thermal damage. The decedent had no medical history of diabetes mellitus, however.

Conclusion/Discussion: This is the first known report of homicide by isopropanol ingestion. In cases of suicidal/accidental isopropanol ingestion concentrations of acetone are much higher than isopropanol due to the shorter half-life of isopropanol versus acetone. Here, a low concentration of acetone was found in the blood and none in the urine. The blood level of nifedipine was below toxic level despite the presence of numerous tablets within the stomach; these findings suggest a quick demise due to isopropanol ingestion. In addition, alcohol dehydrogenase preferentially oxidizes ethanol over isopropanol; this would have extended the half-life of isopropanol, thus increasing its toxicity.

PLATFORM

S13: Ivabradine, a Non-Fatal Cardiotoxant Overdose and Its Presence in an Unexplained Case

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Background/Introduction: Ivabradine was approved in 2015 for the treatment of inappropriate sinus tachycardia and other electrophysiological disorders. It has a unique mechanism of action on the sinoatrial (SA) node of the heart (natural pacemaker) where it antagonizes or has a negative chronotropic effect on the funny channel (I_f) when I_f is in the open state, resulting in slowing of the heart rate. Ivabradine is only selective to the SA node and does not target the atrioventricular (AV) node or neurohormonal system, as other medications do (anti-arrhythmic, beta and calcium channel blockers). In an ivabradine overdose, an individual will not go into cardiac arrest but will experience bradycardia. The slowest an individual's heart rate can reach is ~30 – 60 bpm since only the SA node is affected. Presented is a method and determined plasma/serum concentrations of ivabradine in two cases. The first case is an intentional overdose case involving ingestion of fifty 5mg pills. On arrival, vital signs and neurologic exam were unremarkable. Within 30 min, her heart rate decreased to 31 bpm, but remained normotensive with no change in mentation. Two additional bradycardic episodes occurred, but were treated. Thirty-six hours post ingestion, her heart rate was 67 bpm. The second case involved an individual who presented with symptomatic sinus bradycardia over 1-2 weeks. She was previously prescribed ivabradine for sinus tachycardia, which she reportedly had not taken for several days. The bradycardia persisted with heart rates in the 40-bpm range, but responded to exertion. Due to a subsequent event with a syncopal episode, she was hospitalized for 3 days. Ivabradine had a reported half-life of 2 – 11 h, her clinical presentation indicated its presence, reported use did not.

Objective: To develop and validate a method for the analysis of ivabradine in overdose and unexplained cases.

Methods: Ivabradine and its isotopically labeled standard (50 ng/mL) were extracted using a 1 mL aliquot of plasma/serum and extracted with 4 mL of ethyl acetate, vortex mixed for 3 min, and centrifuged at 8000g for10 min. The organic layer was removed and evaporated under a stream of nitrogen gas in a dry-bath at 40℃. The residue was dissolved in 200 mcL of mobile phase, vortex-mixed for 1 min, and centrifuged at 8,000g for 5 min. then transferred to the LC-MS/MS system where 5 mcL was injected for analysis. Linearity was assessed from 1 to 100 ng/mL. Validation controls were prepared at 1, 5, 30, 75, and 750 (dilution 1:9) ng/mL Storage stability was determined at 5, 30, and 75 ng/mL for 1 and 2 days, and 1 month at 5 ℃, benchtop for 72 hours and 3 freeze-thaw cycles.

Results: The overdose patient's plasma/serum ivabradine concentration was determined in three different blood collection tube types (EDTA, serum separator and plasma separator) upon arrival and at 3 h post-admission. The arrival concentrations were 525, 464, and 460 ng/mL, respectively, and 3 h post-admission concentrations were 130, 120, and 110 ng/mL, respectively. The unexplained patient's serum ivabradine concentrations at admission, 12 h and 36 h post-admission were 27, 11, and 6 ng/mL, respectively. Intra and inter-run precisions were < 15%, determined bias was < 7%, and QC was stable at all testing conditions.

Conclusion/Discussion: The ivabradine concentrations determined in the overdose patient and unexplained patient were approximately 50 and 2 times the expected therapeutic concentration, respectively. The method was robust and reliable for the analysis of ivabradine in serum or plasma specimens.

S14: Broad Analyte Screening Based on High Resolution Accurate Mass Technology for Postmortem Forensic Applications

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Introduction: Traditional screening methods in postmortem toxicology have relied on drug class screening utilizing immunoassay-based technology, chromatographic methods, and analyte-specific approaches using LC-MS/MS or GC-MS with library matching. Limitations of these methods include non-specific identifications, time-consuming development and validation, and limitations in analyte scope. Application of high-resolution accurate mass (HRAM) technology enables a screening method that allows broad analyte scope, fast development and validation, and robust identification of analytes. The developed method is based on a combination of targeted and unknown analyte matching, using data dependent MS^2 (ddMS^2) collection.

Objectives: Develop an analytical method based on HRAM and library matching to enhance screening capabilities for post-mortem drug identification.

Methods: Blood and serum samples were prepared by phospholipid depletion (Phree, Phenomenex) and a dilution protocol for urine. Analysis is carried out with a Thermo Scientific Vanquish UPLC and Q Exactive Orbitrap Mass Spectrometer equipped with a biphenyl column (2.1 X 50 mm, 2.6 µm). The method was validated by analysis of >150 analytes to determine interferences, carryover, limit of identification, and identification accuracy (or measurement of error). Internal standards are used to evaluate extraction efficiency with an RSD limit of <20%. Parallel studies were conducted in whole blood, serum, and urine and the results were verified with an LC-QqQ screening and confirmation methods. The resulting data is matched to a verified library with known performance which includes matching parameters on retention time, isotope pattern matching, and exact parent and fragment masses. Matching is also conducted with an in-house modified library based on the Thermo Scientific MZ Vault spectra program.

Results: Sample preparation is carried out with the Phenomenex Phree solid phase phospholipid removal columns by mixing the matrix in a 4:1 ratio with acidified organic buffer and eluting the mixture directly from the column with positive pressure. The samples a dried and reconstituted in methanol for analysis. This system generates percent recoveries of >80% for a broad range of analytes with one sample preparation procedure; simplifying the laboratory workflow process and allowing complete processing in less than 1 hour. The verified library is analyte specific and able to detect and identify analytes with limits of identification of typically 5-10 ng/mL and identification accuracy of >93%. The subsequent data files are matched to a verified library of >150 analytes and an MZ vault library of >1500 compounds covering illicit, novel psychoactive and therapeutic drugs (Figure 1).

PLATFORM

Figure 1. Example of HRAM Library Screen Matching
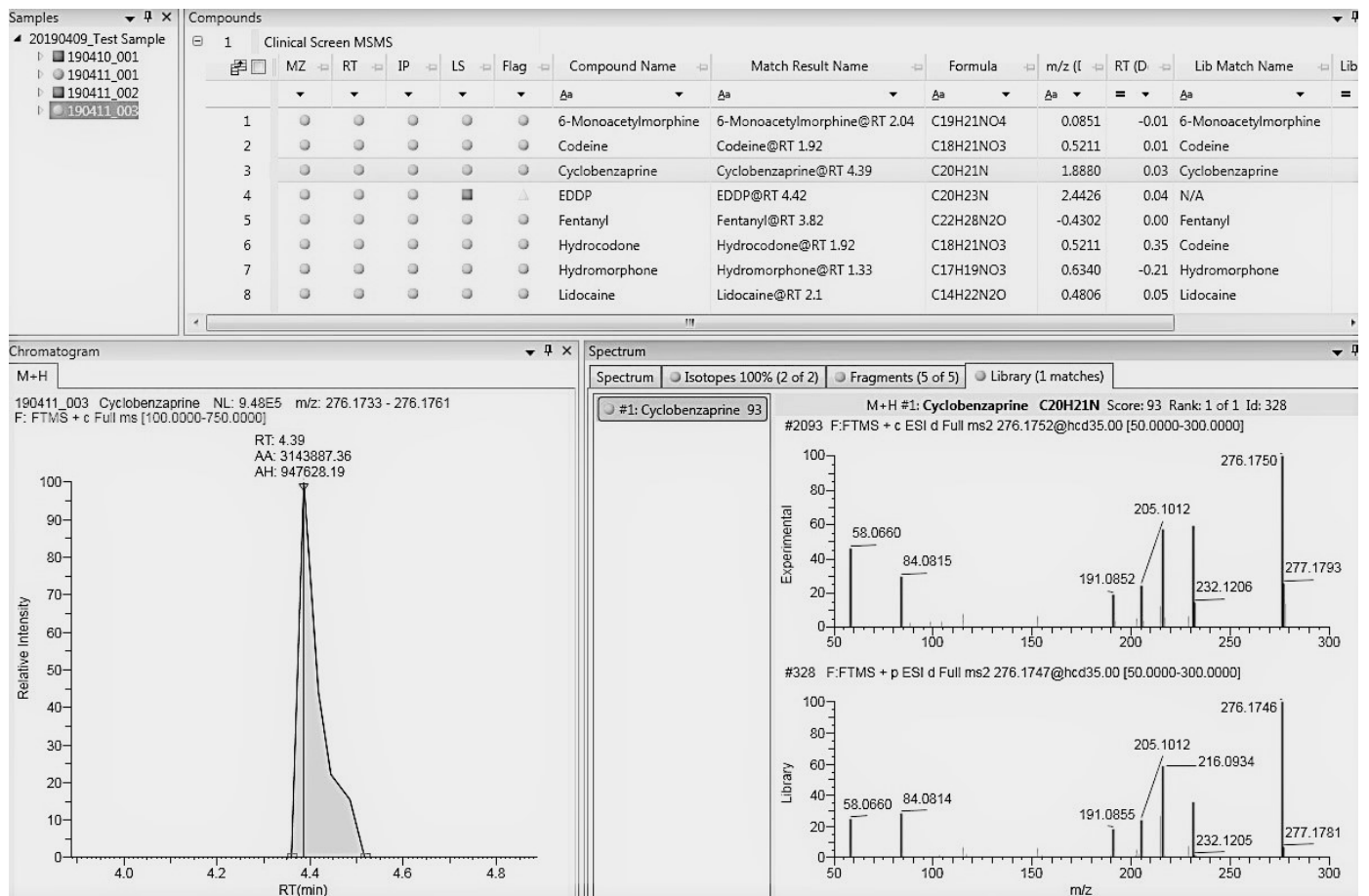
Conclusion/Discussion: We developed a single screening method with a simple sample preparation, a broad scope of analytes, and a fast validation process. The verified and MZ Vault libraries can be expanded in house as additional substances emerge or are added to the market. Positive identification of analytes in patient samples are generated by matching 8 criteria; retention time, peak shape, mass error, isotope pattern matching, number of fragments, library matching, library score, and signal to noise. These criteria have proven to provide high confidence in results.

S15: Identifications and Quantification of Exogenous Insulin Analogs in Postmortem Specimens

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Background/Introduction: The detection of exogenous insulin in postmortem samples is of relevance in that insulin may be implicated in the cause of a death. However, multi-stage sample preparation methodologies not commonly employed in toxicology labs, instability in standard lab ware or specimen containers, and the necessity to analytically differentiate the nearly identical pharmaceutical analogs has remained a challenge for the forensic community. Consequently, the determination of insulin in postmortem cases is not routinely performed.

Objectives: The goal of this project is to develop a straightforward, robust, and forensically valid approach for the differentiation and quantitation of human insulin as well as five pharmaceutical analogs, including insulin Glargine (Lantus®), Glulisine (Apidra®), Lispro (Humalog®), Aspart (NovoLog®), Human (Humulin®), and Detemir (Levemir®) in post-mortem vitreous humor.

Methods: Robotic immunoaffinity extraction was performed on the Agilent AssayMap Bravo automation system using 200 μL of vitreous humor. LC-MS/MS analysis on an Agilent 6495 triple quadrupole mass spectrometer coupled with a 1290 series UHPLC measured insulin β-chains to unequivocally differentiate each analog. Chromatographic separation was performed using an Agilent RRHD 300Å SB-C18 1.8 μm 2.1 x 50mm analytical column with a stepwise separation at 0.4 mL/min over 9 minutes. Mobile phases consisted of water with 0.2% formic acid and acetonitrile with 0.2% formic acid. Method validation was performed in accordance with SWGTOX guidelines.

Results: All analogs performed within the criteria for acceptable performance. Validation evaluated linear range (500 pg/mL – 25,000 pg/mL), limit of quantitation (500 pg/mL), limit of detection (500 pg/mL for insulin Detemir and 125 pg/mL for all other analytes), accuracy and precision (total difference/CV < 20%), interference, carryover, and stability. To date, this method has been used to test 38 vitreous fluid specimens submitted by medical examiners and other death investigators. Evaluation of the data shows that 29% (n=11) tested positive (n=6 for Lispro; n=3 for Aspart; n=2 for Human Insulin).

Conclusion/Discussion: Overall, this workflow has been successfully developed, validated, and applied to authentic forensic samples. Detailed case histories from several cases demonstrate the utility and accuracy of the method. Ongoing efforts aim to optimize and expand the scope of the assay. This effort has focused on improvements to the extraction so that it can be applied to tissue samples that may contain injection marks as well as post-mortem blood. In addition, C-peptide, as well as Insulin Degludec (Tresiba®), are being added to the scope of the assay.

PLATFORM

S16: Improving Scope and Turnaround Times in Postmortem Casework with a Consolidated Methodology Approach

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Background/Introduction: Systematic toxicological approaches that employ both ideology changes and improvements in instrumentation and sample extraction allow for increased efficiency through lower sensitivities, higher specificity and minimizes resources used. Traditional methods utilizing GC-MS typically require iterations of testing, which hinder productivity and turnaround times, particularly for polypharmacy cases frequently seen in modern postmortem toxicology. This two-part presentation aims to discuss the method development and validation of a LC-MS/MS analytical method and to demonstrate how the transition from classic GC-MS methods enhances postmortem toxicology testing and accurate cause and manner of death decisions.

Objectives: The objective of this project was to develop a LC-MS/MS method that consolidates the scope of seven legacy methods for better sensitivity, higher throughout, quantitation of drugs of abuse with minimal sample consumption, and incorporation of smart automated processing for improved quality assurance.

Methods: One hundred microlitres of blood or urine were rapidly extracted using a simple acetonitrile protein crash and subsequent in-vial filtration (Thomson Instrument Company, New Jersey, US) with nitrogen dry-down and starting conditions reconstitution. The LC-MS/MS system was a Sciex Nexera X2 LC-30 with a Sciex QTRAP 6500+ mass spectrometer utilizing an Ion Drive™ Turbo V electrospray ionization (ESI) source in positive mode. Chromatography utilized a gradient on a Kinetex Phenyl Hexyl 100 Å LC Column (100 x 4.6 mm, 2.6 μm) column (Phenomenex, California, US). Data acquisition and processing with customized query for automation incorporating quality assurance was performed. The analytical method was fully validated to SWGTOX and international guidelines including: Bias (Accuracy) and Precision; Dilution Integrity; Carryover; Interferences; Selectivity; Limit of Detection (LOD); Limits of Quantitation; Matrix Effects; Processed Sample Stability; and Linearity experiments. To demonstrate applicability, the described method was applied to previous proficiency test samples and the blood samples of the last 12 months of authentic casework analyses were compared to the scope and limits of detection from the laboratory's legacy methods.

Results: This analytical method incorporates 55 analytes and the customized query facilitates rapid and consistent application of acceptability criteria for data processing and review. This method analyzed 1389 samples (858 blood and 531 urine). There were 2551 analytes (average of 3.0 per sample) and 1938 analytes (average of 3.6 per sample) detected in blood and urine, respectively. Of the top 5 in blood being methamphetamine, amphetamine, morphine, benzoylecgonine, and cocaine, with similar findings in urine. Due to the narrower scope and decreased sensitivity of the seven legacy GC-MS methods, at least 41% of results would have been missed if indeed all seven GC-MS methods were employed for each sample which was atypical. Furthermore, 11% of results were not within the previous scope of our analytical methods.

Conclusion/Discussion: Historically, the San Francisco Office of Chief Medical Examiner relied heavily on a GC-MS testing regime, comprised of individual drug-class confirmation and quantitation assays, typically requiring iterations and prioritization of testing given insufficient sample volumes. The analytical method presented here significantly increases the efficiency and throughput for postmortem casework. Cases containing any of the top 5 analytes would have typically required three distinct GC-MS assays, 3 mL of blood, and upwards of 30 hours of active staff time. The described LC-MS/MS analytical approach mitigates the need to perform multiple assays, utilizes only 0.1 mL of sample, and reduces the extraction time from 16 hours to 3.5 hours. Incorporation of 10 additional analytes, including prescription medication and opioids, allows for a more comprehensive testing regime to better inform pathologists for cause of death determinations. This method promotes consistency and standardization in quality assurance while increasing efficiency in case management and decreasing turnaround times, pivotal in modern postmortem toxicology.

PLATFORM

S17: Comparison of Spectrophotometric Methods for the Determination of Carboxyhemoglobin in Post-Mortem Blood.

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Background/Introduction: Carbon monoxide (CO) poisoning presents an interesting challenge for post-mortem toxicology laboratories. The co-oximeter, a device based on spectrophotometric principles and developed for % carboxyhemoglobin (COHb) determination, became extremely popular in post-mortem laboratories; it provides simple and quick sample preparation along with instant results while removing the need for any deep understanding of the principles underlying its function. One brand of co-oximeter, Instrumentation Laboratory (IL), became particularly popular. A recent survey of post-mortem laboratories (n=17) indicated a majority currently use or formerly used IL co-oximeters for their CO testing. Recently, the IL brand has been discontinued and less technical support is offered. Laboratories, therefore, need to find alternative solutions. One particularly appealing alternative included spectrophotometric methods which would boast similarly simple and quick sample preparation procedure while at the same time offering enough precision and accuracy for post-mortem work. A major hurdle for laboratories is the selection of the appropriate spectrophotometric method to determine %COHb, as a variety of procedures have been published over the years.

Objective: The objective of this work was to compare four spectrophotometric methods for determining %COHb using bias, precision, sensitivity, ease of analysis and the effect of potential interferences commonly observed in postmortem samples.

Methods: The four methods were chosen based on literature findings and/or the appropriateness for postmortem testing. These methods are based on (A) relating %COHb to the ratio of COHb and Hemoglobin (Hb) absorbance of a reduced sample, (B) multi-component analysis of all hemoglobin species, (C) multicomponent analysis of a reduced sample and (D) derivative spectroscopy. All samples were analyzed with the same conditions using an Agilent CARY 60 Spectrophotometer with instrument control and data processing performed using Cary WinUV Software. Samples were prepared according to the specifications given in published papers that effectively used the method in question for spectrophotometric analysis. Postmortem samples that previously tested positive for CO were utilized for analysis in addition to blood samples that were prepared by bubbling CO into human blank blood.

Results: Methods B and C boasted similar within-run and between run-precision (<5%) with methods A and D being notably less precise at lower concentrations but still within lab standards for %CV (<20%). In terms of bias, all methods produced results with acceptably low bias (<20%) with methods B and D being below 5%. In terms of dynamic range, all methods were linear (r^2 >0.99) at low range (~ 3 % COHb) up to 100% COHb except for method D, which had notable discrepancies at low %COHB (<10%) and extremely high %COHB (>70%). There was variation across methods, but general agreement within 10% COHb for the majority of post-mortem samples analyzed. There were a few slight trends, such as method B producing lower values and method D producing higher %COHb than expected. Samples exposed to excessive heat showed no major trends across methods. Decomposed samples, on the other hand, produced more accurate results using method B, with methods C and D having moderately over-calculated %COHB values (47-53% error) and method A's over-calculations appearing more extreme (~70% error). In terms of ease of analysis (sample preparation time, data analysis etc.), method B is by far the simplest with methods C and D being the most involved.

Conclusions/Discussion: While the methods performed similarly in terms of typical validation requirements, the ability to more effectively deal with decomposed samples and simple sample preparation made method B (multicomponent analysis) most suitable for post-mortem testing.

Keywords: Carboxyhemoglobin, Post-mortem, Hemoglobin, Co-Oximetry, Spectrophotometry, Carbon Monoxide

PLATFORM

S18: Rapid Method by LC-QTOF-MSE for the Addition of Prevalent Synthetic Cannabinoids in a Forensic Toxicology Laboratory

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Background/Introduction: Synthetic cannabinoids (SCs) are one of the largest groups of novel psychoactive substances being monitored by the Drug Enforcement Administration (DEA) (DEA Emerging Threat Report, 2018). It was reported in 2016, that over 240 SCs have been identified worldwide (Ambroziak and Adamowicz, 2018). The abuse of these compounds poses a serious threat to the public health, health care systems, and both forensic and clinical toxicology laboratories. Similar as to what has been reported nationally by several organizations, Washington DC has experienced spikes in emergency calls due to SC usage. The emergence of new synthetic cannabinoids requires both a prompt response and a selective method that allows for the detection of prevalent SCs.

Objectives: Create a rapid and selective analytical screening method for multigenerational SCs in blood that would enable a forensic toxicology laboratory to better respond to a public health crisis.

Methods: Extraction optimization was conducted using liquid-liquid extraction on drug free antemortem and postmortem blood fortified with the analytes of interest. The parameters evaluated were extraction solvent ratio, buffer pH, as well as the optimal volume for both sample aliquot and reconstitution. Based on optimization results, sample preparation was conducted via a liquid-liquid extraction with 1.0 mL blood extracted into 4 mL 80:20 hexane/ethyl acetate and 1 mL 0.13M sodium borate buffer (pH 9.3). Samples were then mixed for 10 minutes on the Orbital Mixer, sonicated for 5 seconds to remove emulsions, and centrifuged at 3,000 rpm for 5 minutes. The upper organic layer was immediately transferred to clean conical tubes and evaporated at 40 °C under nitrogen. Following evaporation, samples were reconstituted in 50 µL of 50:50 mobile phases, vortex mixed, and transferred to labeled autosampler vials for analysis.

Analyses were carried out using a Waters Acquity Ultra pressure liquid chromatograph (UPLC) paired with a Waters Xevo G2 mass spectrometry-quadrupole time of flight detector. Reference standards purchased from Cayman Chemical (Ann Arbor, MI, USA) were utilized for chromatographic optimization to evaluate the following parameters: analytical column, injection volume, gradient and source optimization, reconstitution volume, and chromatographic separation. Each parameter was evaluated based on retention time, fragment ions, and detector counts. Per the results of the optimization experiments, separation was performed on a CORTECS UPLC C18 (1.6 µm, 2.1 mm X 100 mm) analytical column using a 5 mM ammonium formate in water (A): 0.1% formic acid in acetonitrile (B) with a flow rate of 0.5 mL/min and a run time of 11 minutes.

Data were processed using Waters UNIFI Scientific Information Software in data independent acquisition mode. The processing method consisted of a compound library of 36 compounds and 6 internal standards, each having an expected retention time, expected neutral mass, and expected fragment ions. The criteria for identification was mass error <2 ppm, RT error <0.35 min, the identification of the expected fragment ions, and peak areas >200 counts.

Results: A compound database library was created for 36 prevalent multi-generational synthetic cannabinoids in the District of Columbia. The method allowed for the simultaneous detection and identification of all 36 analytes. With the discovery of new compounds, this method allows for the rapid analyte addition of new analytes and identification based on accurate mass, retention time, and fragment ions.

Discussion/Conclusion: An LC-QTOF method was developed for the simultaneous detection of 36 synthetic cannabinoids in antemortem and postmortem blood. This method utilizes a library database for rapid screening of synthetic cannabinoids and allows for library expansion when new SCs emerge, providing a sensitive and specific analytical screening method to meet public health needs.

Keywords: synthetic cannabinoids, blood, LC-QTOF-MS[E], optimization, library

PLATFORM

S19: Validation of a Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) Method for the Quantification of 13 Designer Benzodiazepines in Postmortem Blood

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Background/Introduction: The misuse of designer benzodiazepines, as an alternative to prescription benzodiazepines and for drug-facilitated sexual assaults, has emerged as a growing threat, due in part to the ease of purchasing these drugs on the internet. Causing concern for safety, is the lack of dosage information resulting in users self-medicating, often leading to unintended overdoses, coma, or death at higher doses.

Objectives: With limited published data regarding the quantification of designer benzodiazepines in forensic cases, the main objective was to develop and validate a method for the determination of thirteen designer benzodiazepines in postmortem blood, to add to the in-house method that already included a limited number of common designer benzodiazepines.

Methods: The developed method consisted of analysis for 3-hydroxyphenazepam, clobazam, clonazolam, delorazepam, deschloroetizolam, diclazepam, flualprazolam, flubromazepam, flubromazolam, flunitrazolam, meclonazepam, nifoxipam, and pyrazolam in 0.5 mL postmortem blood using LC-MS/MS. The analytes were treated with solid phase extraction before undergoing separation on a column and analyzed on the mass spectrometer in electrospray positive mode using multiple-reaction monitoring. The parameters tested for method validation consisted of linearity, bias, precision, limit of quantitation (LOQ), limit of detection (LOD), matrix effect, carryover, stability, interference, and dilution integrity. The validated method was then applied to blood specimens from the New York City Office of Chief Medical Examiner.

Results: The linear range of the calibration curve was 1-200 ng/mL, and up to 500 ng/mL for 3-hydroxyphenazepam, clobazam, flubromazepam, and pyrazolam. The limits of detection and quantitation were 0.5 ng/mL (signal/noise (S/N)>3) and 1 ng/mL, respectively. The calculated bias, intra-day imprecision, relative standard deviation (RSD), and inter-day imprecision RSD were ± 12%, 3-20%, and 4-21%, respectively. Matrix effects ranged from -52% to 33% with RSD values ranging from 3-20%, indicating consistent effects throughout multiple sources. Recovery ranged from 35-90%, where only two compounds were below 50%. Carryover was not present in samples with concentrations up to 1000 ng/mL. Dilution integrity was maintained for all compounds, except diclazepam in the 1:5 dilution and nifoxipam in the 1:2 dilution. Interferences caused by endogenous and exogenous compounds were not observed regarding the identification of the target compounds. Upon storage in the autosampler for stability studies, nifoxipam and flubromazepam displayed deterioration, while clonazolam, deschloroetizolam, diclazepam, flualprazolam, and flunitrazolam quantified greater than 25% of the original concentration after 24 h. Of the 33 blood specimens that were re-analyzed using this method, five samples tested positive for designer benzodiazepines consisting of clonazolam, delorazepam, diclazepam, flualprazolam, and flubromazolam, with concentrations from 0.93 to 68.91 ng/mL.

Conclusion/Discussion: The validated method consists of the simultaneous determination of 13 designer benzodiazepines in blood using solid phase extraction (SPE) and a 13.5-minute analysis on the LC-MS/MS, proving to be simple, reproducible, sensitive, and robust.

Keywords: Designer Benzodiazepines, LC-MS/MS, Blood

PLATFORM

S20: 5F-MDMB-PINACA and 5F-MDMB-PICA Metabolite Identification and Cannabinoid Receptor Activity

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Introduction: According to the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA), there were 179 synthetic cannabinoids reported as of 2017. In the United States, there were 24,501 identifications of synthetic cannabinoids reported to the National Forensic Laboratory Information System (NFLIS) in 2017. The synthetic cannabinoid, 5F-MDMB-PINACA or 5F-ADB, accounted for 28% of those identifications. The synthetic cannabinoid, 5F-MDMB-PICA, is structurally similar to 5F-MDMB-PINACA with an indole group replacing the indazole. While very little is known about 5F-MDMB-PICA, its pentylfluoro side chain like that of 5F-MDMB-PINACA, indicates high potency at the cannabinoid 1 (CB1) receptor. Limited data exist from *in vivo* or *in vitro* metabolic studies of either of these synthetic cannabinoids, so potential metabolites to identify use may be missed.

Objectives: To investigate metabolism of both 5F-MDMB-PINACA and 5F-MDMB-PICA utilizing human hepatocyte incubations to identify *in vitro* metabolites. To examine authentic case specimens that involved these synthetic cannabinoids in order to verify metabolites identified by hepatocyte incubations. Also, to identify the potency and efficacy of 5F-MDMB-PINACA and 5F-MDMB-PICA by examining activity at the CB1 receptor.

Methods: 5F-MDMB-PINACA and 5F-MDMB-PICA were incubated with pool human hepatocytes (20-donor). Ice cold acetonitrile was used to end incubations after 1, 3, and 5 h. An Agilent 1290 infinity ultra-high performance liquid chromatography system coupled with an Agilent 6550 iFunnel quadrupole time-of-flight (QTOF) mass spectrometer equipped with a Dual Agilent Jet Stream electrospray ionization source was used for the analysis. Data from hepatocyte incubations and urine samples were processed using a Personal Compound Database and Library (PCDL) generated in-house using MassHunter PCDL that included common and expected phase I and phase II metabolites. Postmortem (n=3) and antemortem (n=1) urine samples were analyzed with and without hydrolysis.

Analysis of receptor activation was carried out on aequozen recombinant CHO-K1 cell lines expressing the human CB1 receptor using a coelenterazine based luminescence assay and a Spark 10M plate reader. Dose response curves were prepared in triplicate and compared to those of JWH-018.

Results: Biotransformations found in this study included phase I transformations (amide hydrolysis, carboxylation, dehydrogenation, ester hydrolysis, N-dealkylation, hydroxylation, oxidative defluorination, oxidative defluorination to pentanoic acid, propionic acid formation at the indole/indazole side chain) and phase II transformations (glucuronidation). A total of 21 5F-MDMB-PINACA metabolites (A1 to A21) were identified with 3 compounds unique to urine specimens. From hepatocyte incubations and urine samples, 22 metabolites (B1 to B22) were identified for 5F-MDMB-PICA in this study. Phase II metabolites (glucuronides) were identified in 5F-MDMB-PINACA (n=5) and 5F-MDMB-PICA (n=3). Receptor activation studies concluded that 5F-MDMB-PINACA and for 5F-MDMB-PICA were equally potent as JWH-018 at the CB1 receptor.

Discussion/Conclusion: For both compounds, ester hydrolysis and ester hydrolysis in combination with oxidative defluorination were the most abundant metabolites produced *in vitro*. At least one of these biotransformations were present in each of the case samples presented and are in agreement with previous literature. Both 5F-MDMB-PICA and 5F-MDMB-PINACA were found to be active at the CB1 receptor with potency similar to JWH-018.

Keywords: 5F-ADB, 5F-MDMB-PICA, Hepatocyte Metabolism, Synthetic Cannabinoids, CB1 activity

PLATFORM

S21: Quantitation of Tianeptine and its Metabolite in Blood and Urine by LC-MS/MS

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Background/Introduction: Tianeptine (*Stablon, Coaxil, or Tatnol*) has been prescribed throughout countries in Europe, Asia, and South America as an antidepressant since the late 1980's. However, within the United States, its use has not been approved by the U.S. Food and Drug Administration, but it can be readily purchased online as a dietary supplement. While structurally similar to tricyclic antidepressants (TCAs), tianeptine has a unique mechanism of action by enhancing the reuptake of serotonin. Studies have also shown that tianeptine is a mixed mu- and delta-opioid receptor agonist that is less potent than morphine. Its active metabolite, MC5, is formed from extensive b-oxidation and has a similar potency to its parent compound at mu-opioid receptors but little activity at delta-opioid receptors. These characteristics have led to recent increased misuse and abuse across the United States.

Objectives: The objective of this project was to develop and validate a quantitative method for the analysis of tianeptine and its active metabolite, MC5, in blood and urine specimens using liquid chromatography tandem mass spectrometry (UPLC-MS/MS). Case samples, tentatively identified to contain tianeptine, were then analyzed for quantitative confirmation using the validated method.

Methods: Method validation was conducted according to the Scientific Working Group for Toxicology (SWGTOX) guidelines. Parameters assessed included linearity, limit of detection (LOD), limit of quantitation (LOQ), extraction recovery, precision, bias, dilution integrity, carryover, stability at 3 temperatures (-20°C, 4°C, 23°C), interferences, and ionization suppression/enhancement. To 200 µL aliquots of matrix, 25 µL of deuterated internal standard for each analyte was added followed by 3 mL of phosphate buffer (pH 6). Samples were then subjected to a solid phase extraction optimized using 130 mg Clean Screen® DAU extraction columns (UCT). Following elution and evaporation, samples were reconstituted in initial mobile phase conditions for analysis using a Waters Acquity I Series UPLC® coupled to a Waters Xevo® TQD mass spectrometer. Chromatographic separation was achieved on an Acquity UPLC® BEH C18 column (2.1 x 100 mm, 1.7 µm) with 5 Mm ammonium formate pH 4 and 0.1% formic acid in methanol using gradient elution and a flow rate of 0.45 mL/min.

Results: All validation parameters were deemed acceptable based on SWGTOX guidelines. Baseline resolution was achieved for both analytes in under 4 minutes. Linearity was determined to range from 25-1000 ng/mL using 6 points of calibration. LOD and LOQ were set to 25 ng/mL. Extraction recovery was 60% for both analytes. Inter- and intra-run precision ranged from 1.6 to 8.6%. Bias was calculated to be ≤ 6% for both analytes. Dilution integrity was evaluated at 1:2, 1:10, and 1:40 and had no significant impact on accuracy of results. No interferences were observed from the matrices tested or from commonly encountered drugs of abuse, and no carryover was detected in blank samples run immediately after injecting the highest calibrator (1,000 ng/mL). Both analytes were stable in blood and urine at all temperatures for 30 days. Six blood and three urine samples were analyzed using this method. Concentrations ranged from 620-10,000 ng/mL with a median of 1,000 ng/mL for tianeptine, and 1,300-12,000 ng/mL with a median of 1,900 ng/mL for MC5 in blood. In urine, concentrations ranged from 2,700-28,000 ng/mL with a median concentration of 12,000 ng/mL for tianeptine while all cases were >40,000 ng/mL for MC5.

Conclusion/Discussion: The method was optimized and fully validated for the quantitation of tianeptine and its active metabolite MC5 in human blood and urine. To our knowledge, this is the first method that quantitates both tianeptine and MC5 in these two matrices using UPLC-MS/MS technology in forensic casework. The method was successfully applied to the analysis of 9 samples from 5 cases and generated quantitative results which will aid in the interpretation of future cases.

PLATFORM

S22: Method Development and Validation of U-Type Opioid (Utopioid) Series Compounds and their Identification in Authentic Case Samples

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Background/Introduction: U-Type Opioid ("Utopioid") series compounds were originally developed in the 1970s by the Upjohn pharmaceutical company as opioid analgesics. Despite their intended medical use, they were never studied in humans. Beginning in 2015 however, compounds from this series (e.g. U-47700) began to appear in forensic casework. Since 2015, additional compounds from the series have proliferated, creating analytical challenges due to the occurrence of several isomers, especially ring substitution, and their chemically similar nature. Differentiation of isomeric species is unachievable solely by mass spectrometry techniques due to same chemical formulae and their very similar fragmentation patterns. This necessitates chromatographic separation for definitive identification of these compounds. On the current illicit market, there are two specific sets of utopioid isomers: U-48800/U-51754 and U-49900/Propyl-U-47700/Isopropyl U-47700 that exemplify this challenge.

Objectives: The purpose of this presentation is to describe an analytical method capable of chromatographically resolving the isomeric species within the utopioid class of compounds. In addition, we discuss the concentrations of the drugs found in authentic biological specimens (blood and/or urine).

Methods: A method was developed and optimized using a Waters Xevo TQ-S Micro tandem mass spectrometer coupled with a Waters Acquity UPLC® (Milford, MA). Chromatographic separation was achieved using a gradient elution on a Waters Cortecs® UPLC® C18+ column (2.1 mm x 100 mm, 1.6 um), heated to 60°C with a flow rate of 0.3 mL/min. The mobile phases used for analysis were 5 mM ammonium formate (pH=3, MPA) and 0.1% formic acid in acetonitrile (MPB).

The following utopioid analytes were included in this method: 3,4-methylenedioxy-U-47700, U-50488, 3,4-ethylenedioxy-U-51754, 3,4-ethylenedioxy-U-47700, U-48800, U-47700, U-49900, propyl-U-47700, *N*-desmethyl-U-47700, U-69593, U-51754, *N,N*-didesmethyl-U-47700, 4-phenyl-U-51754, isopropyl-U-47700 and U-47931E (bromadoline).

Blood samples (0.5 mL) were extracted using solid phase extraction (SPE) with 130 mg UCT Clean Screen® DAU extraction columns. SPE columns were conditioned and washed before eluting with ethyl acetate, acetonitrile, and ammonium hydroxide. Samples were evaporated to dryness and reconstituted in mobile phase.

Results: The method was validated according to ASB guidelines, evaluating the following criteria: bias, intra- and inter-assay precision, linearity, limit of detection (LOD), limit of quantitation (LOQ), matrix and commonly encountered interferences. All isomers were successfully resolved chromatographically.

U-48800 cases (n=10) were detected by NMS Labs (Willow Grove, PA) from January to December 2018, submitted from Florida (n=4), Pennsylvania (n=3), Ohio (n=1), Minnesota (n=1) and South Carolina (n=1). Case histories were provided with the corresponding concentrations for two cases. Case 1: a 46 year-old female was found in the supine position on the bedroom floor with heroin packets stamped with a blue skull wearing a hat and the words "Stranger Danger". The concentration of U-48800 was 5.3 ng/mL, in addition to other opioids and common drugs of abuse found. Case 2: a 41-year-old male was found deceased in the bathroom of a hotel room. Drug paraphernalia was found as well, with the heroin packet having the same stamp and markings as Case 1. The concentration of U-48800 was <1 ng/mL, in addition to fentanyl and cocaine present.

Seven U-49900 cases were detected by NMS Labs from April 2017 to January 2018 from the following states: Illinois (n=3), Georgia (n=1), Missouri (n=1) and Pennsylvania (n=1). All cases were male subjects and U-49900 was found in combination with novel opioids.

Conclusion/Discussion: The utopioid series creates analytical challenges for forensic analysis, specifically due to isomeric pairs. The ten cases testing positive for U-48800 in this study screened positive for U-51754 during the initial analytical testing. Without definitive separation of the utopioids, U-48800 would not have been confirmed in these cases. Forensic laboratories should be aware of the utopioid series and develop methods for their identification and confirmation.

Keywords: U-series, Postmortem, LC-MS/MS

S23: Evaluation of Synthetic Cannabinoid Metabolites in Human Blood in the Absence of Parent Compounds

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Background/Introduction: Synthetic cannabinoids present unique challenges for detection in toxicological samples compared to other classes of novel psychoactive substances (NPS). Historically, synthetic cannabinoid testing has consisted of targeted assays for parent compounds in blood and metabolites in the urine; however, few laboratories evaluate the presence of metabolites in blood. Following internal[1] and external[2] reports of metabolites present in blood in the absence of parent compounds, our laboratory re-evaluated our analytical approach to assess the stability of synthetic cannabinoids in blood under various storage conditions and the prevalence of metabolites only in authentic forensic toxicology cases.

Objectives: The objective of this study was to determine the stability of 5F-ADB (5F-MDMB-PINACA), FUB-AMB (MMB-FUBINACA), ADB-FUBINACA, and 5F-MDMB-PICA in human whole blood. 5F-ADB, FUB-AMB, and 5F-MDMB-PICA all have terminal methyl esters, a moiety hypothesized to be more susceptible to breakdown or conversion. These four analytes represented the most commonly encountered synthetic cannabinoids in the United States in 2018 and early 2019. Additionally, the prevalence of these parent compounds and/or their metabolites was investigated.

Methods: Preserved human whole blood was fortified with 5F-ADB, FUB-AMB, ADB-FUBINACA, and 5F-MDMB-PICA at 10 ng/mL. Blood aliquots were stored at room temperature (n=3), refrigerated (n=3), and frozen (n=3) for 1, 2, 3, 7, 14, 21, and 35 days (total n=27). At the given intervals, blood samples were prepared for analysis by liquid-liquid extraction. Blood samples (0.5 mL) were acidified with 5% phosphoric acid in water (1 mL) and extracted into a mixture of hexane, ethyl acetate, and methyl tert-butyl ether (3 mL, 80:10:10 v:v). Extracts were analyzed via a Sciex TripleTOF® 5600+ quadrupole time-of-flight mass spectrometer coupled with a Shimadzu Nexera XR ultra high performance liquid chromatograph (LC-QTOF) using SWATH® non-targeted acquisition. The stability of each analyte was determined by monitoring change in peak area ratio over time. In addition, blood extracts were obtained from NMS Labs for analysis by LC-QTOF using the same method used for monitoring stability.

Results: FUB-AMB was found to be highly unstable, with no parent compound detectable after only 1 day when stored at room temperature and after only 3 days when stored refrigerated. 5F-ADB was also found to be highly unstable, with noticeable loss in parent compound (up to 90%) after 7 days when stored at room temperature or refrigerated. 5F-MDMB-PICA was found to be the most stable of the methyl esters; however, more than 75% of the parent compound was lost after 30 days.

The degradation of all three compounds (5F-ADB, FUB-AMB, and 5F-MDMB-PICA) corresponded to an increase in their respective butanoic acid metabolites: 5F-ADB 3,3-dimethylbutanoic acid, FUB-AMB 3-methylbutanoic acid, and 5F-MDMB-PICA 3,3-dimethylbutanoic acid. This indicates that the presence of these metabolites in blood samples may be due, at least in part, to instability. Since March 2018, 193 toxicology cases were positive for 5F-ADB 3,3-dimethylbutanoic acid (n=114) and FUB-AMB 3-methylbutanoic acid (n=102). Of these cases, 83 blood samples (43%) were positive for synthetic cannabinoid metabolite(s) in the absence of the parent compound. FUB-AMB 3-methybutanoic acid was detected with the highest frequency without FUB-AMB (n=53), consistent with the results of the stability study. 5F-ADB 3,3-dimethylbutanoic acid was detected without 5F-ADB in 43 blood samples. To date, we have not identified 5F-MDMB-PICA 3,3-dimethylbutanoic acid in the absence of 5F-MDMB-PICA in blood samples.

Conclusion/Discussion: 5F-ADB, FUB-AMB, and 5F-MDMB-PICA were found to be unstable in preserved human whole blood, based on storage conditions. These analytes were found to degrade to their respective butanoic acid metabolites. Laboratories should analyze for synthetic cannabinoid metabolites in blood in addition to the parent compounds, as these findings are toxicologically significant.

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PLATFORM

S24: Development, Optimization and Validation of Criteria for the Identification of over 700 Drugs and Metabolites by LC-QTOF/MS with SWATH Acquisition

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Background/Introduction: In comprehensive toxicological drug screening, robust identification criteria is necessary to ensure that all drugs of abuse, therapeutics, poisons and novel psychoactive substances are correctly identified. It is pivotal for any laboratory routinely investigating increasing numbers of deaths, drug facilitated crimes (DFC) and drug impaired driving (DUID) casework to efficiently process results and thus, to significantly mitigate the amount of resources required to produce high quality reporting in medico-legal settings. Over 700 compounds, including anesthetics, analgesics, anticonvulsants, antidepressants, antihistamines, antipsychotics, barbiturates, benzodiazepines, cannabinoids, cathinone, cocaine and metabolites, fentanyl, hallucinogens, opioids, phenylethylamines, new psychoactive substances (NPS), and nonsteroidal anti-inflammatory drugs (NSAID), were analyzed by ultra-high-performance liquid chromatography (UHPLC) coupled with a quadrupole-time of flight mass spectrometer (QTOF/MS) system, a high-resolution mass spectrometry (HRMS) technology that utilizes sequential window acquisition of all theoretical fragment-ion spectra (SWATH). Variations in criteria for mass error, retention time, percentage of isotope ratio difference, and library hit scoring parameters along with percentage allocations of these parameters were optimized to determine a threshold for combined weight scores. The scores within individual parameters also provided further insight to the identification confidence. The system was designed as an automated workflow with pre-set criteria for inclusion, consideration and exclusion.

Objectives: To optimize and validate the identification criteria system that obtained the highest efficiencies allowing for the accurate screening of casework by HRMS.

Methods: A simple, nonspecific sample preparation technique was employed. The UHPLC system consisted of an ExionLC coupled with a SCIEX LC-QTOF/MS X500R system (AB Sciex LLC, Framingham, MA, USA). Separation was performed using a Phenomenex Biphenyl (50 x 4.6 mm, 2.6 µm) at a flow rate of 1.2 mL/min. The 700 compounds were prepared using certified reference materials purchased from Cerilliant (Round Rock, TX, USA), Cayman (Ann Arbor, MI, USA) and Lipomed (Cambridge, MA, USA). Limits of Scope (LOS) for each compound were determined based on satisfactory signal to noise (S/N) ratios and integration review. LOS mixes were then prepared and extracted in blood in triplicate over 3 days as recommended in the SWGTOX guidelines. Additionally, settings were varied within each of the four identification criteria parameters (mass error, retention time, percentage of isotope ratio difference, and library hit score) and various weight allocations for the combined weight score were evaluated to optimize the system for efficiencies, sensitivities, and specificities.

Results: The most favorable system was selected for the greatest number of compounds. Customized rules were then determined for compounds not exhibiting targeted efficiency with the generalized criteria. The efficiency, sensitivity, specificity and positive predictive value (PPV) of the software's native *traffic light* system was then determined. Finally, an applicability validation study was performed using authentic case specimens and proficiency test samples providing further feedback regarding accurate identification of analytes.

Conclusion/Discussion: The identification criteria determined was optimized for efficiency in the setting of the sample matrix. This subsequently allowed for the *traffic light* system and a combined weight score objectively that mitigates false negative results in an automated workflow solution for high throughput forensic toxicology laboratories. The described HRMS method provides an efficient, comprehensive, and accurate toxicological testing regime for immediate reporting in postmortem, DUID and DFC casework received by the San Francisco Office of the Chief Medical Examiner.

S25: Structural Identification of Metabolites of Synthetic Cannabinoids JWH-018, AM-2201, THJ-018, THJ-2201 and 5F-AKB-48 Using In-house Synthesized Standards, Hepatocytes & LC-QTOF-MS

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Background/Introduction: Synthetic cannabinoids are quickly metabolized and often, only the metabolites can be identified in urine. Therefore, the elucidation of metabolites and the production of metabolite reference materials is especially important.

These metabolites are mainly identified through metabolism studies using either *in vitro* models such as liver microsomes, cell lines or hepatocytes, or authentic urine samples. Metabolites are identified using high resolution LC-MS. While high resolution LC-MS is a powerful analytical technique, it is limited in its ability to distinguish structural analogs, such as hydroxyl group position.

Objectives: The aim of this study was to produce or obtain reference materials for synthetic cannabinoid metabolites and identify the exact structure of the metabolites produced by hepatocytes with a special focus on those that cannot be unambiguously identified by their MSMS-spectra.

Methods: Thirty-one metabolites of JWH-018, AM-2201, THJ-018, THJ-220 and 5F-AKB48 were synthesized in-house. They were characterized by LC-MS and NMR. Nine metabolites from the same parent analytes were obtained from commercial sources.

For JWH-018, the 2-, 4- and 5-OH-pentyl, 5-, 6- and 7-OH-indole, 4-OH-naphtyl, 2,3- and 4,5-diOH-pentyl, as well as the pentanoic acid were available. For AM-2201 the 2-, 3- and 4-OH-pentyl, as well as 6-OH-indole were available. For THJ-018 the 2-, 3-, 4- and 5-OH-pentyl, 2,3- and 4,5-diOH-pentyl as well as the pentanoic acid were available. For THJ-2201 the 2-, 3- and 4-OH-pentyl were available. For 5F-AKB48, 2-, 3- and 4-OH-pentyl, as well as 4eq- and 4-ax-OH-adamantyl were available. Metabolites from 5-fluorinated-analogs were also compared to potential non-fluorinated metabolites. As such, for AKB48, N-dealkylation alone and in combination with 4-ax-, 4-eq- and tert-OH-adamantyl, 5-OH-pentyl alone and in combination with 4-ax- and 4-eq-OH-adamantyl, pentanoic acid alone and in combination with 4eq-, 4ax- or tert-OH-adamantyl, were also included.

The parent drugs were incubated with cryopreserved hepatocytes (5 µM, 1 million cells/ml, 5 h). After stopping the reaction with acetonitrile, the supernatants were analyzed by LC-QTOF-MS (Agilent 6550) using mobile phases consisting of ammonium formate and acetonitrile on an HSS T3 column (Waters).

Hepatocyte metabolites were matched with reference materials based on retention time, mass, and when available, MSMS-spectra.

Results: After incubation with the non-fluorinated cannabinoids JWH-018 and THJ-018, 4-OH-pentyl metabolites were observed. Similarly, after incubation with the fluorinated analogs AM-2201 and THJ-2201, 5-OH-pentyl metabolites were observed. In addition, for AM-2201, the pentanoic acid metabolite was observed.

For 5F-AKB48, the major monohydroxylated metabolite was 5-OH-pentyl, but 4eq-OH-adamantyl was also observed together with the corresponding dihydroxylated metabolite (5-OH-pentyl,4eq-OH-admantyl), as well as with the pentanoic acid metabolite. In addition, a metabolite matching the reference materials of both pentanoic acid in combination with 4eq-OH-adamantyl and pentanoic acid in combination with tert-OH-adamantyl was observed.

Conclusion/Discussion: When analyzing a series of structural analogs, chromatographic separation is particularly important. In this study, most analogs were well separated but still, one of the metabolites had two co-eluting structural analogs. As reference material for both 5F-AKB48 pentanoic acid,tert-OH-adamantyl and 5F-AKB48 pentanoic acid, 4eq-OH-adamantyl were available it became apparent that they could not be differentiated. That said, 4eq-OH-adamantyl seem to be the preferred isomer for other metabolites, which were separated from their tert-OH-adamantyl counterparts.

This study provides new insights into the preferred sites of metabolism of cannabinoid analogs that could be used in future metabolism studies, as well as for the synthesis of reference materials. This is especially true for 5F-AKB48, where 4eq-OH-adamantyl appears to be a preferred site of metabolism.

PLATFORM

S26: 4F-MDMB-BINACA: An Emergent Synthetic Cannabinoid Implicated in Forensic Toxicology Casework

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Background/Introduction: As the number of emergent opioids and fentanyl analogues has been decreasing, the number of new synthetic cannabinoids has increased again within the last year. 5F-MDMB-PICA and 4F-MDMB-BINACA have recently emerged and become more prevalent as the positivity of 5F-ADB and FUB-AMB have declined. Of concern to forensic toxicologists is the prevalence of synthetic cannabinoids in medicolegal death investigations (MDI) and driving under the influence of drugs (DUID) investigations. Synthetic cannabinoids present analytical challenges as a result of their complex and diverse chemistries, specifically issues involving recovery of parent compounds and metabolites from biological samples, unknown metabolic profiles, and need for high sensitivity. This presentation focuses on 4F-MDMB-BINACA, one of the most prevalent synthetic cannabinoids in use in the United States in 2019 and an analyte that may not be widely incorporated into forensic toxicology testing protocols.

Objectives: This study sought to characterize the emergence and prevalence of 4F-MDMB-BINACA in forensic toxicology casework. In addition, the *in vivo* metabolism of 4F-MDMB-BINACA was investigated by analysis of authentic casework samples to identify metabolites for incorporation into blood and urine testing protocols.

Methods: Extracts of blood and urine samples, correlating to cases of suspected synthetic cannabinoid use, were obtained from NMS Labs for re-analysis at CFSRE for sample mining, a process that allows for discovery of analytes not targeted within the initial scope of testing. Extracts were analyzed using a Sciex TripleTOF® 5600+ quadrupole time-of-flight mass spectrometer coupled with a Shimadzu Nexera XR ultra high performance liquid chromatograph. SWATH™ acquisition was used for isolation of product ions following the acquisition of precursor ions by TOF MS scan. This non-targeted analytical approach allows for complex drug characterization and novel psychoactive substance (NPS) discovery.

Results: 4F-MDMB-BINACA (Figure 1) was first identified in a forensic toxicology blood extract through sample mining in January 2019, following receipt of standard reference material and incorporation into the library database. Subsequently, data mining of previously acquired data revealed that the first analytical detection was in November 2018, around the same time as first identification in national seized drug reports. To date, 4F-MDMB-BINACA ingestion has been identified in 54 cases where blood or urine extracts were received for LC-QTOF testing (roughly 5,000 extracts have been received since early 2018). Cases were submitted from 13 states (TX [n=14], IN [n=14], PA [n=10], UT [n=3], FL [n=3], and AR, CT, NY, MI, KS, LA, and CO [n=1]) and the District of Columbia (n=3). The majority of cases were MDI (n=37), but cases were also submitted from DUID investigations (n=12) and with unknown circumstances (n=5). Forty-four of the individuals were male and 4 were female; sex in 6 cases was unknown. 4F-MDMB-BINACA was found in combination with 5F-MDMB-PICA (35%) and APP-BINACA (15%), another emergent synthetic cannabinoid.

The *in vivo* metabolism of 4F-MDMB-BINACA was investigated based on the previously reported metabolism of 5F-ADB and 5F-MDMB-PICA using a data mining approach. This resulted in the identification of 9 metabolites, discovered using four representative urine samples and four representative blood samples. The two major metabolites identified were 4F-MDMB-BINACA 3,3-dimethylbutanoic acid and 4-OH-MDMB-BINACA (Figure 1). Further data mining revealed the presence of 4F-MDMB-BINACA 3,3-dimethylbutanoic acid in 9 total cases from blood (n=6) and urine (n=3) samples. Urine results were found in the absence of parent compound.
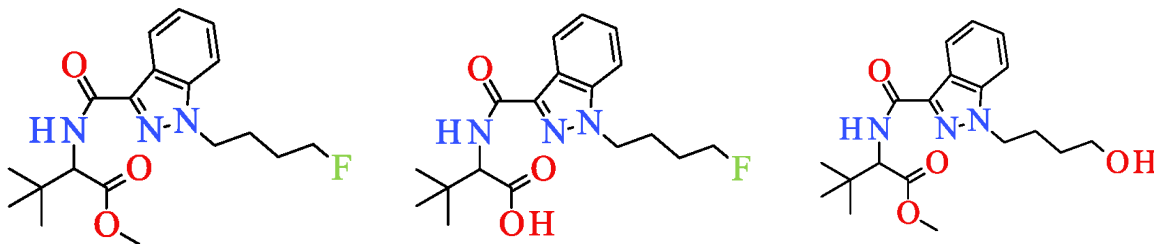

Figure 1: Structures of 4F-MDMB-BINACA (left), 4F-MDMB-BINACA 3,3-dimethylbutanoic acid (middle), and 4-OH-MDMB-BINACA (right)

Conclusion/Discussion: Forensic toxicologists should be aware of the emergent synthetic cannabinoid 4F-MDMB-BINACA, as its popularity continues to increase. In May and June of 2019, 4F-MDMB-BINACA was the most prevalent synthetic cannabinoid detected based on our findings. Laboratories should consider addition of 4F-MDMB-BINACA to blood testing procedures and 4F-MDMB-BINACA 3,3-dimethylbutanoic acid to urine testing procedures. Sample mining and data mining procedures for the discovery of drugs and/or NPS missed during standard targeted analysis should be more widely implemented.

S27: Designer Benzodiazepine Positivity in Toxicological Casework: 2012-2018

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Background/Introduction: Designer benzodiazepines (DBZD) are rapidly increasing as drugs of abuse; typically repurposed from pharmaceutical development, they have appeared in online shops as "research chemicals" or clandestinely sold in counterfeit pharmaceuticals. Such substances are potentially more harmful than pharmaceutical benzodiazepines, having unknown pharmacological/toxicological profiles and have added to the complexity and dangers of the illicit market for benzodiazepines. Phenazepam and etizolam were some of the first DBZD to appear in the illicit drug market in the US. Other compounds that have been reported from this class include flubromazepam, flubromazolam, delorazepam, diclazepam and clonazolam.

Designer benzodiazepines are agonists at the $GABA_A$ receptor and potentiate the inhibitory action of gamma-amino butyric acid (GABA). Benzodiazepines as a class of CNS depressants have anxiolytic, sedative-hypnotic, muscle relaxant, and anticonvulsant properties, and are often prescribed as anxiolytics, anesthetic adjuncts, and treatment for obsessive-compulsive disorders. The phenyl group appears to be a requirement for benzodiazepine activity; structural modifications of the benzodiazepine structure affects both the potency and duration of action.

Objectives: This presentation describes the positivity of DBZD, a class of novel psychoactive substances (NPS), in forensic toxicology casework between 2012 and 2018.

Methods: Testing for DBZD has evolved over time in response to the emergence of a substance, in addition to improvements in testing capabilities. Screening for DZBD typically occurs using Liquid Chromatography/Time of Flight Mass Spectrometry (LC-TOF/MS). Confirmatory analysis for phenazepam and etizolam were conducted by Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) but initial results were reported qualitatively. Subsequently, a quantitative LC-MS/MS panel for 11 DBZD was developed and validated. Reporting limits range from 2 ng/mL (etizolam, flubromazolam), 5 ng/mL (clonazolam, diclazepam, delorazepam, meclonazepam, and bromazepam) to 20 ng/mL (flubromazepam and phenazepam). Between 2012 and Q1 2019, data from routine toxicological testing, including death investigation and driving under the influence (DUI) casework, was compiled to investigate positivity rates of DBZD in blood specimens.

Results: Between 2012 and 2017, there were 58 qualitative phenazepam confirmations in blood. Etizolam was added to the scope of testing in 2015; 94 qualitative results were reported between 2015 and 2017.

A directed quantitative assay for 11 DBZD was launched in October 2016, with all blood confirmations migrated to the quantitative test by January 2018. Between 2016 and Q1 2019, a total of 742 positive DBZD results were reported in 609 blood samples under the quantitative procedure. Etizolam accounted for 49% of the positive findings (98.8% were reported at or above the reporting limit of 2 ng/mL). Other positive findings were as follows: flubromazolam (n=110), delorazepam (n=103), diclazepam (n=57), bromazepam (n=40), flubromazepam (n=34), clonazolam (n=29), phenazepam (n=4) and meclonazepam (n=2). 14.4% cases reported more than 1 DBZD; 10 cases reported more than 4 DBZD, with one case reporting 6 different substances. A common finding is a combination of delorazepam and diclazepam (n=34), since diclazepam metabolizes to delorazepam, which then metabolizes further to lorazepam, which is also available by prescription.

In 2018 alone, DBZD were confirmed in 45 blood samples obtained from driving under the influence casework; 23 of those cases involved etizolam and 13 included flubromazolam.

Conclusion/Discussion: DBZD are a group of NPS that are routinely abused; their appeal lies in possessing the same effects as benzodiazepines such as alprazolam or diazepam, but typically circumventing routine drug testing. Many laboratories have limited testing capabilities for DBZD, but proper investigation may help assist in identifying cases in which DBZD may be present. DBZD have cross-reactivity with commercial benzodiazepine immunoassays; unconfirmed immunoassay screens should be evaluated for the presence of DBZD. It is important for forensic laboratories to include designer benzodiazepines within their scope of testing, especially for laboratories that perform testing for DUI cases.

PLATFORM

S28: Casework Involving the Designer Benzodiazepine Clonazolam in Northern Virginia

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Background/Introduction: Clonazolam is a novel designer benzodiazepine that bears structural resemblance to clonazepam, but modified with a triazolo ring moiety. In recent months, the northern Virginia (NoVA) area has seen a number of cases involving this drug. As there is minimal literature available on the toxicology of clonazolam, it is currently difficult to assess the impact of toxicological findings involving this drug.

Objectives: This presentation describes an investigation initiated to better understand and characterize the toxicological impact of clonazolam on human performance and postmortem casework in the NoVA area. Presented findings will include quantitative determinations of clonazolam and its metabolite, 7-aminoclonazolam. These findings will also be correlated to corresponding observations and additional toxicological findings in casework.

Methods: Analysis of clonazolam and 7-aminoclonazolam was achieved using solid phase extraction. Briefly, samples were buffered using 0.1 M phosphate buffer (pH 6) and added to prepared SPE cartridges (UCT CleanScreen ZSDAU020). SPE columns were prepared through washes of methanol and 0.1 M phosphate buffer (pH 6) prior to sample addition. After sample addition, columns were washed with water and 0.1 M acetate buffer (pH 4), then eluted with methanol followed by column drying and further eluted with 78:20:2 v/v/v dichloromethane:isopropanol:ammonium hydroxide. Combined eluents were acidified using 0.2% HCl in isopropanol and evaporated to dryness under nitrogen. Samples were reconstituted in mobile phase (98:2 v/v water:methanol with 0.1% formic acid) and analyzed via LCMSMS using multiple reaction monitoring (MRM). The calibration range for clonazolam and 7-aminoclonazolam was 0.00025 – 0.015 mg/L. Numerical values obtained using this method were considered semiquantitative as the method was not fully validated according to SWGTOX guidelines for quantitative determinations.

Results: Specimens of whole blood submitted for toxicological testing were determined to have clonazolam concentrations ranging from 0.0019 – 0.013 mg/L. Each case positive for clonazolam was also identified as containing 7-aminoclonazolam. Numerous cases among those analyzed were determined to involve poly-drug use. The most commonly co-detected drugs included THC and its metabolite (THC-COOH; 71%) and alprazolam (29%). Demographics associated with clonazolam use are displayed in the table below:

Age	% of cases		Race	% of cases		Gender	% of cases
15-20	64		White	57		Male	57
21-25	14		Black	21		Female	36
26-30	7						
30+	0						

The final presentation will contain updated information as additional data are collected.

Conclusion/Discussion: The data collected during this investigation has given insight into the toxicological impact of the potent designer benzodiazepine clonazolam. Results indicate that clonazolam concentrations in the µg/L range are potentially toxicologically relevant. This suggests a potency approximately an order of magnitude higher than other benzodiazepines commonly encountered in casework (e.g., alprazolam, clonazepam, lorazepam). Side effects associated with clonazolam use appear to be consistent with typical benzodiazepine toxicopharmacology: sedation, lethargy, incoordination, slowed reactions, and slurred speech. While polypharmacy was present in many of the cases investigated, the overall circumstances of the cases demonstrate side effects that are more severe than the other toxicological findings would generally be expected to elicit, demonstrating the impact of clonazolam. Another interesting facet of this research were the statements made to law enforcement of having consumed other benzodiazepines (i.e., alprazolam, clonazepam). While it is difficult to parse out the veracity of statements made by those under investigation for illegal activity, it does suggest the potential for unknowing consumption of a more potent benzodiazepine than the user intended.

PLATFORM

S29: A Portrait of GHB Consumption, One of the Four Most Prevalent DUID Findings in Québec (Canada)

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Background/Introduction: In 2008, a modification to the Criminal Code introduced the International Drug Evaluation and Classification Program (DECP) in Canada to address the problem of driving under the influence of drugs (DUID). Under this program, suspected impaired drivers are arrested and undergo a 12-step evaluation that ends with a biological sample collection. Toxicological analysis of these samples throughout the years revealed a widespread prevalence of gamma-hydroxybutyrate (GHB) in cases from the province of Québec. GHB is a depressant of the central nervous system (CNS) known to impair driving ability. It can be prescribed to treat narcolepsy and other sleep disorders (Xyrem®) or taken as a drug of abuse (*juice*, *liquid E*).

Objectives: DECP cases analyzed in 2018 in the province of Québec (Canada) were reviewed to determine the demographics of GHB abuse and driving.

Methods: Biological samples (98.7% urine) were stored at 4°C pending completion of toxicological analyses. Following extraction by protein precipitation, samples were submitted to a targeted screening by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS 5500 QTRAP®, Sciex). 144 compounds were analyzed quantitatively or qualitatively in this method, including GHB. Confirmation and quantification of GHB was carried out by gas chromatography coupled to mass spectrometry (GC-MS) on samples derivatized with BSTFA+TMCS (99:1, Cerilliant). If relevant, general unknown screening was carried out by solid phase extraction (Oasis® HLB, Waters) followed by gas chromatography coupled with mass spectrometry and a nitrogen-phosphorous detector, which has a selective sensitivity for nitrogen and phosphorus containing compounds (GC-MS/NPD, 7890/5975 MSD, Agilent). All quantitative methods were validated under ISO 17025:2005, CAN-P-1578 and SWGTOX guidelines. To ensure positive reports of only exogenous GHB consumption cases, the positivity threshold was set at 25 mg/L in urine and 10 mg/L in blood. Data were compiled for all 2018 DECP cases, including gender and age of the suspect, time and location of the event, and detected drugs.

Results: In 2018, GHB was the fourth most common finding in Québec's DECP cases, following methamphetamine, cannabis and cocaine. Out of the 790 samples collected and analyzed that year, 21% (n = 162) tested positive for GHB. Prevalence varied within the 17 administrative regions of the province, ranging from 0% to 38%. Younger males (16-34 years old) accounted for 30% of all GHB cases, an overrepresentation with regards to the general population (14%) similar to the one found in all DUID arrests (41%). Toxicological analyses revealed that co-consumption of GHB with other psychoactive substances was the norm, with only 6 cases (3.7%) where no other psychoactive substance was found. The level of co-consumption for this substance is greater than for overall DECP cases (76%). In that single year, 24 cases (15%) were the result of repeat offenders, with one individual being caught 4 times just in the first half of the year.

Conclusion/Discussion: Abuse of the CNS depressant GHB causes euphoria, relaxation, drowsiness and sedation, which impairs driving ability. Data presented here show a surprisingly elevated GHB prevalence amongst impaired drivers of the province of Québec (Canada). These data correlate well with seized drugs analysis by Health Canada's Drug Analysis Service, which shows that GHB is one of the top five controlled substances identified in Québec but no other province. To our knowledge, no other jurisdiction worldwide has reported such a widespread presence of GHB in forensic toxicology casework. However, several laboratories do not routinely screen for GHB in DUID cases, assuming negligible prevalence. This work highlights the imperativeness of including GHB in routine screening in selected jurisdictions where the consumption habits warrants it.

PLATFORM

S30: Buprenorphine Prevalence in DUID Cases in Southwest Virginia

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Background/Introduction: Buprenorphine is a semisynthetic mixed agonist/antagonist opioid that is 25 - 40 times stronger than morphine, effective by non-parenteral administration and has a long half-life. These properties make it an effective drug to treat opioid dependence. The current opioid epidemic has made buprenorphine drug dependence therapy common and its detection in driving-under-the-influence of drugs (DUID) cases frequent. Buprenorphine was recently promoted to a Tier I drug in the 2017 Update of the Recommendations for Toxicological Investigation of Drug-Impaired Driving and Motor Vehicles Fatalities.

Objective: To investigate buprenorphine and norbuprenorphine blood concentrations, other drugs detected with buprenorphine, compare blood concentrations with driving performance, behavior, and field sobriety tests in DUID cases in Southwestern Virginia.

Methods: The Virginia Department of Forensic Science Western Toxicology Laboratory began confirmation and quantification of buprenorphine in blood for DUID cases in August 2017. Cases that screened presumptive positive for buprenorphine by ELISA using Immunalysis reagents were extracted with UCT ZSDAU020 SPE columns and quantified using an Agilent 1290 liquid chromatograph and 6460 tandem mass spectrometer. The method was validated according to SWGTOX guidelines. Case histories were collected from evidence submission documents that contained officer notes or interactions between officer, attorney and toxicologist at court.

Results: The linear range for buprenorphine and norbuprenorphine is 0.5 to 20 ug/L. 111 cases of buprenorphine and 110 cases norbuprenorphine quantified within the linear range. Blood buprenorphine concentrations ranged from 0.54 to 9.3 ug/L and norbuprenorphine concentrations ranged from 0.55 to greater than 20 ug/L. The median buprenorphine blood concentration was 1.9 ug/L and norbuprenorphine 2.5 ug/L. The median buprenorphine to norbuprenorphine ratio was 0.7 (range 0.1 – 5.8). Buprenorphine was frequently found with other drugs. Only 14 of 111 cases did not contain additional drugs or alcohol that were included in testing panels. The most common drug classes found with buprenorphine were benzodiazepines, amphetamines and cannabinoids.

	Benzo	Opioid	Meth/Amp	Coc/BE	THC/THCA	Ethanol	Others	None
Cases	61	5	41	6	22	4	20	14
%	55.0	4.5	36.9	5.4	19.8	3.6	18.0	12.6

In cases with available history, buprenorphine was frequently prescribed; however, abused buprenorphine by sublingual, insufflation and parenteral routes of administration was also found. Selected case histories will be presented.

Conclusion/Discussion: The detection of buprenorphine in DUID cases requires a sensitive analytical procedure capable of measuring blood concentrations in the 0.50 to 1.0 ug/L range. Approximately one-third of the cases had buprenorphine blood concentrations less than 1.0 ug/L. Reported driving observations include weaving within lanes, crossing center-lines, failure to follow traffic signs and loss of vehicle control. Typical central nervous system depressant effects such as drowsiness, lethargic behavior, slow reaction time and poor coordination were commonly observed along with pinpoint pupils. No correlation between buprenorphine blood concentration and observed symptoms or performance on DRE or field sobriety tests could be determined. The high number of cases with additional drugs detected add to the complexity of evaluating buprenorphine driving impairment. As with most DUID cases, drugged driving impairment with buprenorphine is determined by poor driving performance, driver behavioral observations, performance on DRE and field sobriety tests and supportive toxicology findings.

Keywords: Buprenorphine, DUID, Human Performance

S31: Curious Cases of Cannabis Use in Colorado

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Background/Introduction: At the Colorado Bureau of Investigation, approximately 70% of DUI cases that request drug toxicology screen positive for cannabinoids and approximately 60% confirm positive for delta-9-tetrahydrocannabinol (THC). Colorado voters first legalized medical marijuana in November 2000 and recreational marijuana in November 2012. A permissible inference law of 5 ng/mL THC was signed into law in May 2013 which allowed a jury instruction that a subject at or above this concentration may be considered to be under the influence of one or more drugs.

Due to the nature of Colorado's laws and the readily availability of cannabis products, the potential for chronic users to operate a motor vehicle has increased. As a result, the concentrations of THC, when evaluated in context with case information, may lead to surprising interpretations and jury verdicts.

Objectives: To highlight three cases involving operating a motor vehicle in the State of Colorado with an unusual combination of driving behavior, Standardized Field Sobriety Test (SFST) performance, blood THC results, and jury verdicts.

Methods: Cases were initially screened by ELISA and confirmed utilizing a liquid/liquid extraction and analyzed by LC/MS/MS for the Cannabinoid analytes THC, THC-OH, and THC-COOH. A set of six calibrators from 1.0-50 ng/ml (THC, THC-OH) and 5.0-250 ng/ml (THC-COOH) and 3 controls were concurrently analyzed with all casework. The measurement uncertainty is 19.2%.

Results: Due to the Colorado Expressed Consent Law, each of the three cases had blood samples collected within one hour of the incident time. Case 1 involved a medical marijuana user that last admitted cannabis consumption 1.5 hours prior to the stop for speeding. Case 2 involved a chronic marijuana user for PTSD that last admitted cannabis consumption 5.5 hours prior to the stop for not having license plates. Case 3 involved a driver who was stopped at an intersection and was rear-ended by a driver that suffered serious injuries. The subject in Case 3 claimed no cannabis use or drug use. A summary of the toxicology results and SFST findings are as follows:

Toxicology Results

ng/mL	THC	THC-OH	THC-COOH
Case 1	32	18	186
Case 2	25	9.5	151
Case 3	20	5.9	217

SFST Findings

	Full DRE Evaluation	HGN	WAT	OLS	LOC
Case 1	No	0/6	2/8	1/4	Present - Left
Case 2	No	6/6	1/8	1/4	Not Performed
Case 3	Yes	0/0	0/8	0/4	Absent

The subjects in Cases 1 and 2 were acquitted at trial. There is no legal update for Case 3.

Conclusion/Discussion: These three case studies exemplify the evolving landscape of cannabis use in the state of Colorado. When the toxicology results are evaluated in context with the observed driving behavior and SFST performance, the indications for impairment are not always clear cut. It can be a challenge to assess the impact of marijuana legalization on impaired driving in light of chronic cannabis use.

S32: Buprenorphine in Wisconsin Drivers

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Background/Introduction: As the opioid epidemic continues in Wisconsin and throughout the United States, the use of buprenorphine (BUP) as a form of medically assisted treatment continues to increase. As a result of the increase in detection by laboratories, BUP has been moved from Tier II to Tier I on the recommendation of the National Safety Council's Alcohol, Drugs and Impairment Division. Relatively little information on the observed effects of BUP, outside a laboratory or a controlled driving course, exist in the literature. Opinions of the medical community, however, vary on whether or not BUP can be abused, diverted and cause impairment in drivers. The Wisconsin State Laboratory of Hygiene (WSLH) has seen an increase in the number of driving cases where BUP is listed as a suspected drug and subsequently detected in the blood of impaired drivers. In 2017, norbuprenorphine (NBUP), the pharmacologically active metabolite of BUP, was one of the top 20 most frequently detected drugs in Wisconsin's Operating While Intoxicated (OWI) casework.

Objectives: The WSLH monitored the detection of BUP and NBUP in Wisconsin drivers over a 2 year period. The objectives of this data evaluation were to 1) determine the number of Wisconsin drivers operating under the influence of BUP and/or NBUP; 2) characterize indicators of impairment observed when drivers were under the influence of BUP and/or NBUP; 3) document the frequency of drivers operating under the influence drugs in addition to BUP and/or NBUP.

Methods: Forensic toxicology data from 2012 to 2017 was compiled for Wisconsin drivers operating under the influence of drugs. Data from approximately 19,000 drivers each year was reviewed for cases where BUP and/or NBUP were reported. Drugs reported in addition to BUP and/or NBUP were documented. To characterize the impairing effects of BUP and/or NBUP, police reports and Drug Recognition Expert (DRE) evaluations were obtained for any cases where BUP and/or NBUP were the only drug(s) reported.

Results: A total of 204 individuals (78 females, 126 males) were driving under the influence of BUP and/or NBUP between 2012 and 2017. Three cases from 2018 were included in the data set. Concentrations of BUP and NBUP in whole blood (ng/mL) ranged (mean) from 0.6 to 14 (2.0) and 0.5 to 20 (2.1), respectively. Benzodiazepines, a category of drugs considered to elevate the risk of central nervous system depression when coadministered with BUP, was detected in 70% of the drivers, followed by amphetamine like stimulants (21%), other opioids (20%), ethanol (8%) and cocaine (7%). Only four of the drivers (2%) included in this data set were operating under the influence of either BUP and/or NBUP alone. Physical and behavioral observations made by law enforcement were inconsistent with those typically reported for drivers under the influence of an opioid. Indicators of impairment varied and included a combination of narcotic analgesic, central nervous system (CNS) depressant and stimulant like effects.

Conclusion/Discussion: Poly drug use is extremely prevalent in Wisconsin drivers, so prevalent that only four of the 204 cases evaluated had BUP and/or NBUP as the only drug(s) detected. Law enforcement made contact with three of the four subjects due to either a crash or poor/reckless driving. Poly drug use in drivers makes it difficult to evaluate the impairing effects of a single drug. While the number of cases reported here is limited, the results demonstrate the complex paradigm associated with forensic interpretation of BUP in OWI casework and the frequency of poly drug administration in Wisconsin drivers. Several cases where BUP and/or NBUP were reported in combination with benzodiazepines and amphetamine like stimulants will be discussed.

S33: Everything is Bigger in Texas: A Look at Alcohol-Impaired Driving Trends in the City of Houston (2014 – 2018)

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Background/Introduction: "Everything's bigger in Texas." Unfortunately, this adage holds true for traffic fatalities in the state. Houston is in Texas' largest county, Harris, which in 2017 saw 456 of the state's 3,722 traffic fatalities as reported by the National Highway Traffic Safety Administration (NHTSA). Of those, 44% were classified as alcohol impaired driving fatalities. While Houston's population from 2014 to 2018 remained steady at 2.2 to 2.3 million residents, requests for alcohol analysis submitted to Houston Forensic Science Center (HFSC) for impaired driving offenses doubled.

Objectives: This study examined alcohol analysis results and case information associated with impaired driving arrests in Houston between 2014 and 2018. Numbers of cases, distribution of blood alcohol concentrations, and demographics were examined. These analyses were intended to provide a better understanding of the impaired driver population in Houston and identify trends observed over the past five years.

Methods: The fatal and non-fatal impaired driving cases analyzed by HFSC for ethanol in blood samples with offense dates falling between January 1, 2014 and December 31, 2018 were included. Blood samples were collected by Houston Police Department from drivers as indicated in the laboratory information management system. Cases were analyzed for ethanol by headspace gas chromatography interfaced with dual flame ionization detection. The limit of quantification (LOQ) was 0.010 g/dL and range of linearity was 0.010-0.500 g/dL for ethanol (LOQ was 0.02 g/dL in 2014). Blood alcohol concentrations (BAC) and demographic data including age (<21, 21-44, 45-65, and >65 years), sex, and race/ethnicity were evaluated. Drug screen and confirmatory analyses were performed for cases with BAC <0.10 g/dL and fatality cases, using enzyme-linked immunosorbent assay and gas/liquid chromatography-mass spectrometry; approximately 50% of drug confirmation analyses were performed by external laboratories. Data were analyzed using Microsoft Excel 2016.

Results: Over the five-year period examined, 11,372 blood samples were analyzed by HFSC as part of impaired driving investigations, with a mean (median) BAC of 0.167 g/dL (0.176 g/dL) and age of 36.4 (34) years. Of the suspected impaired drivers, 80% were male and 19% were female, with no significant difference in mean BAC between the two groups (ANOVA; p=0.45). The mean BACs for drivers younger than 21, 21-44, 45-65 and older than 65 were 0.116 g/dL (25% of cases were negative for ethanol), 0.165 g/dL, 0.177 g/dL, and 0.160 g/dL, respectively. Most drivers were White (64%) with a mean BAC of 0.173 g/dL; 20% of drivers were Black (mean 0.144 g/dL), 5% Hispanic (0.176 g/dL), and 3% Asian (0.169 g/dL). Overall mean BAC decreased between 2014 and 2018, from 0.181 g/dL to 0.157 g/dL, and this trend was attributed to the increasing number of cases received wherein no ethanol was detected (ethanol <LOQ), from 15 cases in 2014 to 445 cases in 2018. Drug prevalence in the ethanol-negative cases was examined; the six most prevalent drugs detected in ethanol-negative cases from 2014 to mid-2018 (some confirmation testing still pending) were cannabinoids (39%), phencyclidine (29%), alprazolam/metabolite (26%), cocaine/metabolites (14%), carisoprodol/meprobamate (14%), and hydrocodone (11%).

Conclusion/Discussion: NHTSA reports from 2014 to 2017 showed Texas having the nation's highest number of alcohol-impaired driving fatalities. In Houston, drugged driving is on the rise as well, evidenced by the increased number of cases received that were negative for alcohol but found to contain one or more drugs. Polydrug cases, here defined as samples confirmed to contain two or more classes of drugs, rose from <0.2% of cases negative for ethanol in 2014 to >5% in 2017. Such data can aid in raising public awareness and designing preventative measures to reduce impaired driving in all its forms in the city of Houston.

PLATFORM

S34: Alabama's DUID Oral Fluid Drug Testing Program – One Year Review

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Background/Introduction: Oral fluid (OF) drug testing in DUID cases has many advantages including rapid, non-invasive collection, ability to collect the specimen proximate to time of driving, and the presence of active, parent drug that likely reflects recent drug use. In August of 2018, the Alabama Department of Forensic Science (ADFS) approved three roadside screening devices (i.e. Draeger DT5000, Alere DDS2, Randox Evidence MultiSTAT) and validated in-house oral fluid evidentiary, confirmation methods.

Objectives: Highlight the results from OF DUID cases analyzed from inception thru 2019 and discuss challenges, lessons, and improvement made to the program over the first year of implementation.

Methods: Roadside OF screening conducted by law enforcement to establish probable cause was conducted using Draeger DT5000 or Alere DDS2 devices. Quantisal® OF collection devices with volume adequacy indicators were used for evidentiary collection. OF Confirmation (evidentiary) testing was conducted using two validated methods on an Agilent 6460 LC/MS/MS. The first method identified 20 drugs of abuse following an in-tip Dispersive Pipette extraction (DPX) technique. In the second method, the extraction of six cannabinoids: THC, THC-OH, THC-COOH, CBN, CBD, and CBG were performed by liquid-liquid extraction. OF scope and cutoffs were selected based on NSC-ADID recommendations for toxicological investigations of drug-impaired driving cases. Accompanying blood specimens were screened by Tecan Evo 75 using Immunalysis reagents or a Randox Evidence Analyzer. Blood confirmations were performed by liquid-liquid or solid phase extraction and GC/MS or LC/MS/MS. Positivity rate, median concentration, and prevalence for three of the most commonly detected drugs (i.e. cannabinoids, alprazolam, and methamphetamine) in OF and blood were determined.

Results: Roadside OF screening devices have been purchased by six agencies. During the first year of the program, select law enforcement agencies and DREs were equipped with Quantisal® collection devices. Approximately, 20 OF DUID submissions have been received through May 2019 with 85% of those cases containing both oral fluid and blood specimens. THC was detected at a higher positivity rate (10/10 = 100%, LOD = 0.5 ng/mL) in OF specimens than blood specimens (3/7 = 43%, LOD = 0.5 ng/mL). Median THC concentrations were 3X higher in OF. Methamphetamine had the same positivity rate (8/9 = 89%) between OF (LOD = 20 ng/mL) and blood (LOD = 5 ng/mL). Alprazolam, despite its known low partitioning into the OF, had the same positivity rate in OF (LOD = 1 ng/mL) and blood (LOD = 1 ng/mL). However, OF alprazolam concentrations were 7X lower than blood concentrations.

Conclusion/Discussion: Alabama is the first crime laboratory to implement a statewide OF program by offering approved roadside OF screening devices and confirmation OF testing. The high parent drug positivity rate in OF makes this specimen an attractive option for DUID testing. This is likely due in part to OF collection close to the time of arrest or crash when compared to blood. However, the combined analysis of blood and OF paints the most comprehensive picture of drug use and a potential explanation and cause for impairment at the roadside. We have recently redesigned our DUI biological specimen kits for statewide distribution. These kits will be available in late 2019. At which point, officers will collect two tubes of blood and one oral fluid specimen for all DUI cases.

S35: Detecting Impairment After Cannabis Administration

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Background/Introduction: As cannabis is increasingly being legalized for both medicinal and recreational purposes, there is a critical need to find a marker for cannabis impairment. The pharmacokinetics and associated pharmacodynamics of cannabis administered via vaporization and oral consumption are currently not well understood and need to be better defined to evaluate methods of determining whether or not an individual under the influence of cannabis is impaired. The purpose of this study was to develop valid and reliable measures of impairment among individuals acutely exposed to orally ingested and vaporized cannabis. Data presented will include outcomes from a battery of cognitive and psychomotor performance assessments, field sobriety tests, subjective drug effect ratings, and analyses of cannabinoid and non-cannabinoid candidate biomarkers measured in blood, oral fluid and urine specimens obtained in parallel with pharmacodynamic measures.

Objectives:

1.) Understand the dose effects of acute oral and vaporized cannabis administration on measures of cognitive/psychomotor performance assessments and field sobriety tests.

2.) Understand which biomarkers in oral fluid and blood and which field sobriety tests are, and are not, predictive of impairment on measures of cognitive performance and functioning following cannabis exposure.

3.) Understand the comparative pharmacokinetic and pharmacodynamic profiles of oral versus vaporized cannabis.

Methods: Twenty individuals who had not used cannabis for at least 30 days participated in six, double-blind, experimental sessions that were separated by at least 1 week. Across all six sessions each participant consumed cannabis brownies containing 0, 10, or 25mg THC or inhaled vaporized cannabis containing 0, 5, or 20mg THC. During each session, blood, oral fluid, and urine were collected and subjective, cognitive, and psychomotor effects were assessed before cannabis administration and for 8hrs thereafter. A variety of field sobriety tests were also administered. Blood, oral fluid, and urine were tested for THC and its primary metabolites using targeted LC-MS/MS. Blood samples also underwent an exploratory screen for possible new biomarkers of impairment.

Results: Subjective drug effects were generally dose-orderly within each route of administration with peak effects being lower and delayed after oral ingestion compared to vaporized cannabis inhalation. Oral dosing of 10 and 25mg, and 20mg vaporized THC doses impaired cognitive/psychomotor performance, but 5mg vaporized cannabis produced discriminative drug effects without impairment. Pharmacokinetic measures indicate target compound profiles were also dose-orderly and route dependent. For blood and oral fluid: 1) THC-COOH concentrations were higher after oral consumption compared to vaporized; 2) THC was higher after vaporization. Blood 11-OH-THC concentrations were higher after oral consumption. THC was highest in oral fluid for both administration routes, and the highest levels in blood for vaporization. Neither THC, THC-COOH, 11-OH-THC, CBD, nor CBN were reliable markers of impairment in blood or oral fluid for either route of administration. Field sobriety tests including walk and turn, horizontal gaze nystagmus, one leg stand, and Romberg balance did not reliably discriminate between impaired and unimpaired individuals.

Conclusion/Discussion: The current understanding of pharmacokinetic and pharmacodynamic characteristics of cannabis administered via vaporization and oral consumption is limited. A greater understanding of these parameters will help determine whether or not an individual under the influence of cannabis is impaired. Our work indicates that THC is not a reliable marker of cannabis impairment.

PLATFORM

S36: Quantification of Cannabinoids in Oral Fluid, Breath, and Whole Blood to Evaluate Markers of Recent Cannabis Smoking and Driving Impairment

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Background/Introduction: With legalization of recreational and medical cannabis occurring in 10 and 33 states, respectively, cannabis usage is on the rise. Increased prevalence of cannabis consumption and subsequent intoxication resulting in impaired driving has led to a growing public safety concern. Recent population-based studies examining motor vehicle crash reports regarding cannabis have been inconclusive or shown moderately increased risk of crashing. With legalization comes the responsibility of developing methods to distinguish which markers correlate with recent cannabis use, but not past use, and to determine cutoff levels that correlate with driving impairment. As a result, many states have adopted *per se* driving laws, which range from zero to specific concentration limits (e.g. < 5 ng/mL THC) in whole blood. These laws have numerous caveats, including detection of THC above *per se* cutoffs in blood of chronic cannabis users when not impaired and the challenges of roadside blood collection. THC concentrations can drop up to 90% in the time it takes from when an impaired driver is pulled over to when their blood is drawn. This underscores the need to pursue matrices with less invasive collection, such as oral fluid and exhaled breath.

Objectives: The overall goal of this study, which is funded by California assembly bill 266, is to evaluate the impact of smoking cannabis on driving performance. As part of the larger project, we aim to determine which cannabinoids or metabolites are the strongest markers of recent cannabis use in whole blood, oral fluid, and breath.

Methods: Liquid chromatography-tandem mass spectrometry (LC-MS/MS) assays to measure Δ9-tetrahydrocannabinol (THC), 11-hydroxy-Δ9-THC (11-OH-THC), 11-nor-9-carboxy-Δ9-THC (THC-COOH), 11-nor-9-carboxy-Δ9-THC glucuronide (THC-COOH-gluc), Δ9-THC glucuronide (THC-gluc), cannabinol (CBN), cannabidiol (CBD), cannabigerol (CBG), tetrahydrocannabiverin (THCV), and Δ9-tetrahydrocannabinolic acid A (THCAA) in whole blood, oral fluid, and breath (THC only) were developed and validated. Oral fluid was collected using the Quantisal device and sample preparation of both oral fluid and whole blood used solid phase extraction (Oasis Prime HLB plates, Waters). Breath samples were collected using the SensAbues device and extracted directly from the device with methanol. Cannabinoids were separated on a BEH C18 column and detected by a TQ-S micro tandem MS (Waters), with a total run time of 5 min per sample.

Study participants (n = 184), either frequent or infrequent users, smoked a cannabis joint with THC concentrations of ~0, 6%, or 13% and were monitored for ~6 hours following smoking. For each participant, the following samples were collected over the specified time range: 5 oral fluid, 5 breath, and 9 whole blood. LC-MS/MS measurements were applied to these samples.

Results: Lower limits of quantification for all analytes were either 0.4 or 1 ng/mL in oral fluid, 0.5 to 2 ng/mL in whole blood, and 80 pg/pad for THC in breath. Inter- and intra-day precision assessment demonstrated CVs from 2 – 15% for all analytes in oral fluid. Matrix interferences for all analytes resulted in biases not exceeding ±20%. Patterns of cannabinoids and metabolite concentrations following smoking were evaluated. Sensitivity, specificity, and positive and negative predictive values for each analyte in all three matrices were assessed.

Conclusion/Discussion: Results demonstrate that THC in oral fluid is a promising marker for detection of recent cannabis use. The breath collection device has limitations resulting in reduced sensitivity; consistent detection of THC is not possible beyond 1 hour after smoking. This study is an important step in scientifically determining if specific cutoff concentrations can be validated to support cannabis DUI *per se* laws. The long-term goals of this study are to improve road safety while minimizing persecution of non-impaired cannabis users.

S37: New Benzodiazepine Variants in Recent Casework

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Background/Introduction: Benzodiazepines are among the most potent GABA receptor modulators prescribed for anxiety. The most commonly prescribed benzodiazepines include alprazolam, diazepam, and clonazepam. Dosages and effects of these drugs are well-known, however, within the last year a new group of unapproved benzodiazepines have appeared with potencies and effects that remain largely unknown. While traditional benzodiazepine immunoassays often cross-react with these new benzodiazepine variants, subsequent tandem mass spectrometry confirmations may overlook these compounds if they are not part of the acquisition method.

Objectives: This presentation raises awareness about the new types of emerging benzodiazepines, and the capabilities needed to identify and confirm their presence in toxicology specimens.

Methods: Toxicological screening was performed on blood and/or urine specimens by headspace gas chromatography, immunoassay, and liquid chromatography time of flight mass spectrometry (LC-QTOF/MS). Confirmatory analyses were performed by a 10 minute liquid chromatography tandem mass spectrometry (LC-MS/MS) assay with a limit of detection set at 10 ng/mL. The routine confirmation assay provides quantitative support for 9 parent drugs along with 4 metabolites, while an extended qualitative control monitors for an additional 23 compounds.

Results: We describe selected case reports where QTOF analysis revealed previously unidentified benzodiazepine derivatives, such as flualprazolam, clonazolam, and flubromazepam. In the first case, an intoxicated driver investigation of a 20-year-old female showed no ethanol in blood, but screened presumptive positive for cannabinoids and benzodiazepines. THC and THC metabolites were confirmed in blood along with alprazolam. QTOF analysis also revealed the presence of flualprazolam, a fluorinated derivative of alprazolam which was subsequently confirmed by LC-MS/MS analysis.

In an investigation of a motor vehicle accident involving a 23-year-old-male, the suspect displayed slurred speech and dilated pupils. Much like the first case, alcohol was negative and the immunoassay was presumptive positive for cannabinoids and benzodiazepines. LC-MS/MS confirmed THC-COOH in blood, but the confirmation panel of known benzodiazepines was negative. A QTOF screen showed the presence of flualprazolam in blood, which was qualitatively confirmed by LC-MS/MS.

A third case identified involved a military personnel readiness investigation where an 18-year-old male arrived for duty and was witnessed to be intoxicated. He admitted to taking a Xanax tablet the night before. The immunoassay produced a presumptive positive benzodiazepine response, however alprazolam was not confirmed. A comprehensive QTOF screen revealed the presence of clonazolam in blood. The proposed metabolite 7-aminoclonazolam was synthesized and used as reference standard in urine to confirm clonazolam use by LC-MS/MS.

The final case describes a 21-year-old male who failed to report for morning duty. When contacted by telephone, he was reported to be incoherent with slurred speech. He stated that he had not been recently drinking alcohol. Blood alcohol was confirmed negative and the immunoassay was positive for benzodiazepines, however the confirmation panel was negative. QTOF analysis revealed the presence of delorazepam, alpha-hydroxyetizolam, and flubromazepam, which were confirmed by LC-MS/MS analysis.

Conclusion/Discussion: The emergence of new benzodiazepine derivatives such as flualprazolam, flubromazepam, and clonazolam underscores the need for laboratories to incorporate these compounds and their metabolites into the list of analytical capabilities. In some of the cases mentioned above, the additional benzodiazepines would have been missed without the specificity provided by high resolution mass spectrometry screening and sensitivity of tandem mass spectrometry confirmations. We recommend that presumptive positive benzodiazepine immunoassays should be pursued further especially if the confirmatory analyses are negative, due to the possibility of new and unforeseen derivatives.

PLATFORM

S38: Trends in the Use of Psychoactive Substances Affecting Fitness to Drive in Poland Based on Routine Examination of Drivers in 2010-2018

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Background/Introduction: Driving while under the influence of drugs (DUID) and/or alcohol is still a major safety problem among many countries all over the world. Substances affecting fitness to drive include psychotropic substances of natural and synthetic origin as well as medicines. Cannabis products are the most widespread psychoactive substances (Senna 2010). Stimulants, especially amphetamines and benzodiazepines are also popular (Blencowe 2012). A very large and diverse group of new psychoactive substances (NPS), so-called "legal highs" are spreading among drivers during last few years, reaching 7% drivers presumptive positive (Wille 2018), which correlates with our findings. The most common NPS in Poland in the last year were NEH, MDMB-CHMICA, 4-FMA, 4-CMC.

Objectives: Presentation of trends in use of drugs affecting fitness to drive based upon blood tests of drivers stopped for routine inspection and from road accidents.

Methods: A blood screen for the presence of cannabinoids, amphetamines, cocaine, benzodiazepines and opiates was performed with enzyme-linked immunosorbent assay (ELISA). Coupled techniques (GC-MS and LC-MS/MS) were used for confirmatory analyses.

Results: The collected statistics refer to the period 2010-2018. These included results from 5846 roadside inspections and 3787 cases in which police indicated a road accident occurrence. In almost half of the cases THC was present in the tested blood samples, a trend has been maintained since 2011. The second most popular substance among drivers is amphetamine, detected in about 23% of cases and methamphetamine (about 6% of cases). MDMA, which has had almost disappeared from the drug market for a few years, reappeared in 2014 and its popularity among drivers is growing, exceeding 4% in 2018. Benzodiazepine derivatives (about 3%), especially clonazepam, are also popular among drivers. Since 2013, there has been an increase in number of cases in which NPS have been detected. On the basis of the attached blood protocols, no conclusions can be drawn regarding the performance of the drivers.

Conclusion/Discussion: Cannabis products are still the most popular group of psychoactive substances among drivers. Only three substances: THC, amphetamine and methamphetamine are responsible for 80% positive results of roadside DUI inspections. The stimulants (amphetamines) are particularly dangerous due to the impaired assessment of the driver's own abilities. Our results indicate that amphetamines are involved in greater risk of participating in an accident in comparison to other substances. Cannabis products are also dangerous because of its prevalence. A very large and diverse group of NPS, so-called "legal highs" are spreading among drivers, exceeding 5% in 2015.

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S39: Comparison of Extraction Methods for Novel Synthetic Opioids in Blood

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Background/Introduction: Novel synthetic opioids continue to be reported in acute and fatal intoxications. Several non-fentanyl derived synthetic opioids, including U-47700, AH-7921, and MT-45, are listed as Schedule I controlled substances by the Drug Enforcement Administration. Current reported analytical methods for the detection of these compounds utilize solid-phase extraction (SPE) and liquid-liquid extraction (LLE). Examination of varying extraction methods applied to a comprehensive method to detect these analytes could provide laboratories with alternative extraction methods to suit their needs.

Objectives: This study compares QuEChERS (quick, easy, cheap, effective, rugged, and safe), filter vials, supported liquid extraction (SLE), and LLE techniques to a validated SPE method for the analysis of 7 novel synthetic opioids in whole blood: U-47700, U-49900, U-50488, AH-7921, MT-45, W-18, and W-15. The five extraction methods were compared in terms of matrix effects, extraction recovery, solvent use, time, and cost.

Methods: Matrix effects (post-extraction addition) and extraction recovery were evaluated at a low quality control concentration (0.75 ng/mL, except 2.5 ng/mL for W-18) in different sources of whole blood in duplicate. Cerex® Clin II (3 mL, 35 mg) SPE columns (Tecan, Baldwin Park, CA), Shimadzu Micro Volume QuEChERS kit™ (Shimadzu Corporation, Kyoto, Japan), Thomson eXtreme|FV® (PVDF) filter vials (Thomson Instrument Company, Oceanside, CA), and Biotage® ISOLUTE SLE+ (2 mL) SLE columns (Fisher Scientific, Hanover Park, IL) were used for sample preparation. When performing SPE, both the acidic/neutral drug and the basic drug fractions were collected for analysis. The QuEChERS and filter vial protocols required lower amounts of matrix volume during sample preparation but drug quantity (0.375 ng) remained constant throughout each experiment. Three elution solvents were evaluated for SLE and 3 organic solvents were assessed for LLE. All samples were analyzed using a validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) method on an Agilent 1290 Infinity II LC coupled to an Agilent 6470 triple quadrupole MS [Lowry *Forensic Toxicol* 2019]. Solvent use and cost were calculated for an individual extracted sample. Cost was estimated for consumable materials only, exclusive of analyst time or instrument cost. Time was estimated based on the protocol for the extraction, including sample preparation and time to dry down.

Results: Overall, SPE produced the least ionization enhancement or suppression (±18.0%) when compared to the other techniques (-55.8 – 83.8%). The four alternative extraction methods resulted in higher recoveries for the W-series analytes (23.2 – 84.3%) when compared to SPE (25.3 – 33.7%). Extraction with SLE (ethyl acetate as the elution solvent) and LLE (70/30 n-hexane/ethyl acetate as the organic phase solvent) provided comparable extraction efficiencies to the SPE method. SPE required the greatest amount of organic solvent use (8.1 mL) and total time (160 min) to complete, with the cost of consumables around $4.64 per sample. The QuEChERS and filter vial protocols required minimal organic solvent use, with 0.3 mL and 0.1 mL respectively. The filter vial method was the cheapest to perform ($2.52) and had the shortest extraction time (15 minutes).

Conclusion/Discussion: While SPE might be the better analytical choice in terms of matrix effects and extraction recovery, other methods required lower amounts of organic solvent, were cheaper to perform in terms of cost of consumables, and required less time to complete. Each method analyzed was effective in capturing synthetic opioids at low concentrations and could be beneficial depending on the desires of the laboratory.

Keywords: Novel Synthetic Opioids, Solid-Phase Extraction, Liquid-Liquid Extraction, Supported Liquid Extraction, QuEChERS

PLATFORM

S40: Validation of a High Throughput Screening and Quantification Method for the Determination of Gabapentinoids in Blood Using a Combination of LC-TOF-MS and LC-MS-MS

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Background/Introduction: In recent years, there has been an increasing awareness of gabapentinoid misuse among individuals with a history of polysubstance use. Both gabapentin (GP) and pregabalin (PGL) are understood to potentiate the effects of opioids, with fatalities being reported with increasing frequency.

Objectives: An efficient procedure was validated to screen and quantitate GP and PGL in blood samples using a combination of liquid chromatography time-of-flight mass spectrometry (LC-TOF-MS) and liquid chromatography tandem mass spectrometry (LC-MS-MS). The methods provided a fast and reliable high throughput screening and confirmation testing strategy for the detection of GP and PGL in blood specimens.

Methods: A protein crash procedure using 100 µL of whole blood was validated using SWGTOX guidelines. Reversed-phase chromatographic separations were performed using a gradient with 0.1% formic acid in deionized water and in acetonitrile as mobile phases. Mass spectral data were acquired in ESI+ mode and captured using MassHunter software. The LC-TOF-MS was operated in full scan mode acquiring a mass range of 100-1700 m/z, while LC-MS-MS data was acquired using dynamic multiple reaction mode (dMRM). A total of 1,091 blood specimens were screened for GP/PGL using LC-TOF-MS and all presumptive positives were then re-scheduled for confirmation using LC-MS-MS.

Results: The limits of detection for both analytes on the LC-TOF-MS and LC-MS-MS were 0.5 mg/L and 0.1 mg/L respectively, with the LC-MS-MS method linear from 0.5-50 mg/L. A total of 101 (9.3%) blood specimens screened positive using the developed LC-TOF-MS method for GP while 13 (1.2%) blood specimens screened positive for PGL. Out of all cases that screened positive for GP and PGL, 100% were confirmed positive by LC-MS-MS. Blood concentrations of GP and PGL ranged from < 0.5 – 215 mg/L, and < 0.5 – 32 mg/L respectively. Of the blood specimens that had previously screened negative by LC-TOF-MS, 10% (N=100) were randomly selected and tested by LC-MS-MS with 100% confirmed negative for GP and PGL.

Conclusion/Discussion: It has been shown that the validated methods provide a fast and reliable testing strategy for the detection of GP and PGL in blood specimens when considering the quick sample prep and instrument runtime and reliability of the results. The validated methods provide laboratories with an alternative testing option for screening and confirmation of GP and PGL by LC-TOF-MS combined with LC-MS-MS.

S41: Comprehensive Drug Screening of Whole Blood by LC-HR-MS/MS in a Forensic Laboratory

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Background/Introduction: Many different panels of drugs have been screened for by liquid-chromatography high-resolution mass spectrometers (LC-HR-MS/MS) including some whole blood large panel screens applied to a forensic setting. With the advantages of retroactive analysis for unknown drugs and increased confidence in automated processing capabilities, LC-HR-MS/MS has become a primary candidate for development of front-end comprehensive screening.

Objectives: The Georgia Bureau of Investigation currently performs front-end drug screening of whole blood by a combination of enzyme immunoassay (EIA) and liquid-chromatography tandem mass spectrometry (LC-MS/MS). EIA screens for opioids, cocaine, cannabinoids, barbiturates, and benzodiazepines.   LC-MS/MS analyzes for 180 additional drugs. In an effort to streamline this process and make it more efficient, the creation and validation of a robust method utilizing a Q Exactive Focus ™ high-resolution accurate mass spectrometer was investigated.

Methods: A 0.5 mL aliquot of whole blood is precipitated in a test tube by adding 1 mL of a 75:25 (acetonitrile:acetone) solution while vortexing. After precipitation the samples rest for 5 minutes allowing any emulsions to form. The samples are then vortexed to mix (breaking up any emulsions) and centrifuged for 10 minutes. The supernatant is poured over into a clean test tube fitted with a filtering reservoir. The filtering reservoir is removed, and the supernatant is split into two separate fractions for LC-HR-MS/MS analysis. A 0.1mL aliquot of the precipitate is taken and placed in a test tube and combined with 20 µL of 2% HCl in methanol and dried at 60°C for 3 min., then reconstituted with an 80:20 (H_2O:methanol) solution for a broad positive mode analysis method that looks for 184 analytes including opioids, anxiolytics, antidepressants, anti-psychotics, stimulants, and hallucinogens.   The remaining precipitate is then combined with 2 mL of 0.1N HCl and 3mL of hexanes, vortexed for 30 seconds, and centrifuged for 5 minutes. The hexane layer is transferred to a clean test tube and evaporated to dryness for 5 minutes at 60°C. These samples are reconstituted in a 25:75 (H_2O: methanol) solution for negative/positive switching mode analysis that looks for barbiturates as well as THC and its metabolites.

Separation is conducted on an Agilent Poroshell 120 EC-C18 2.1x100 mm 2.7-micron column with a 4.5-minute gradient for the positive ion method at a 6oo µL/min flow rate and over 8.5 minutes for the positive/negative ion switching method at a 200 µL/min flow rate. Mobile phases for both analysis consist of mobile phase A (0.1% formic acid and ~15 mM ammonium formate in water and mobile phase B (0.1% formic acid and ~15 mM ammonium formate in methanol:acetonitrile 50:50. Data acquisition software was set to produce a mass spectrum once a high resolution mass was detected in a retention time window of 0.2 min.

Results: A qualitative screen for all drugs of interest was developed and subsequently validated. Most drugs demonstrated little to no ion suppression producing LOIs of at least 2 µg/L for most drugs, with parent THC's LOI being 0.5 ng/mL. Interferences of isomers were investigated; the findings demonstrated that although isomers are not always fully resolved, the instruments processing software can determine presence of multiple isomers with the exception of amitriptyline and matrotiline. A 1000 case concordance of postmortem and antemortem whole blood specimens demonstrated that the methods results were equivalent to the LC-MS/MS and EIA results traditionally obtained in case work.

Conclusion/Discussion: We successfully developed and validated a LC-HR-MS/MS method that would provide a more data rich sample set that is amendable to automated processing for the front-end screening of routine toxicology cases.

S42: Determination of 30 Synthetic Cathinones in Postmortem Blood using Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS)

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Background/Introduction: Synthetic cathinones, commonly referred to as "bath salts," are one of the top three new psychoactive substances (NPS) seized in the United States. They are powerful amphetamine-like psychostimulants that also share cocaine-like effects and have been sold as "legal highs." Due to their desired effects, synthetic cathinones have increased in popularity world-wide since 2009. Although self-reported use of these substances has decreased in the past five years, they are increasingly used as adulterants in other recreational drugs such as ecstasy. Since illicit drug manufacturers constantly create new cathinones to evade judicial consequences, difficult challenges arise with the analysis and interpretation of such drugs. To keep up with these new stimulant drugs that continue to appear in the illicit market, up-to-date sensitive and robust methods are needed to reliably detect new cathinone analogs as they emerge.

Objectives: A low-sample-size liquid chromatography tandem mass spectrometry (LC-MS/MS) method was developed and validated to quantify 30 synthetic cathinones in postmortem blood with the application to selected casework specimens from 2015 to 2019 within the NYC Office of Chief Medical Examiner.

Methods: Solid phase extraction (SPE) using 0.25 mL postmortem blood was performed with mixed-mode cation exchange cartridges (Clean Screen® XCEL I, UCT Inc.). After reconstitution, all samples were separated in an Agilent Technologies 1600 Infinity LC equipped with Poroshell 120 EC-C18 column (2.1 mm x 100 mm, 2.7 μm) with 0.1% formic acid in water and 0.1% formic acid in acetonitrile as mobile phases. The total run time was 16 minutes and the flow rate was 0.4 mL/min. All analytes were detected by an Agilent Technologies 6460-triple quadrupole mass spectrometer operating electrospray ionization in positive mode. Two multiple reaction monitoring (MRM) transitions were monitored for each analyte. Ethylone-d5, mephedrone-d3, alpha-PVP-d8, dibutylone-d3 and methylone-d3 were used as internal standards. The method was validated in accordance to SWGTOX guidelines including bias, imprecision, matrix effect, extraction recovery, process efficiency, calibration model, carryover, interferences, stability and dilution integrity.

Results: The linear range of the calibration curve was 1 to 500 ng/mL (R^2 > 0.99) for all compounds with a weighting factor of 1/x (15 compounds) or $1/x^2$ (15 compounds). Both imprecision and bias were evaluated at three different concentrations (3, 30 and 300 ng/mL) in triplicate each of the 5 days (n=15) of validation and met all allowed criteria (CV < 20%, bias 80-120%). No matrix effects were observed with values ranging from -5.1 to 13.3% (CV 11.4-17.5%, n=10). Extraction efficiency (84.9 to 91.5%) and process efficiency (86.1 to 102.6%) were satisfactory, except for 4-chloroethcathinone which was 63.0% and 64.9%, respectively. No carryover after the upper limit of quantification was detected (n=3). Neither endogenous (n=14) nor exogenous (amphetamines, cocaine, opioids) interferences were observed. This method was applied to 17 postmortem cases received between 2015 and 2019. Eight synthetic cathinones were detected: n-ethylpentylone (n=11, 19 to > 500 ng/mL), ethylone (n=3, 25 to > 500 ng/mL), butylone (n=2, 1.4 to 150 ng/mL), dibutylone (n=2, 13 to 486 ng/mL), methylone (n=1, 19 ng/mL), eutylone (n=2, 1.6 to 2.5 ng/mL), 4-chloro-α-PVP (n=1, 7.2 ng/mL) and pentylone (n=1, 3 ng/mL).

Conclusion/Discussion: We developed a sensitive and specific liquid chromatography tandem mass spectrometry method for simultaneous determination of 30 synthetic cathinones in whole blood, employing 0.25 mL of matrix and achieving a 1 ng/mL limit of quantitation. To our knowledge, this method is the most comprehensive methodology for the determination of up-to-date synthetic cathinones currently available in whole blood. Eight different synthetic cathinones were detected in selected postmortem cases within the past four years, showing a wide range of concentrations from 1.4 to > 500 ng/mL.

PLATFORM

S43: Retrospective Datamining of Novel Opioids Using Previously Acquired Data by Liquid Chromatography Time of Flight Mass Spectrometry (LC-TOF-MS)

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Background/Introduction: Novel psychoactive substances (NPS) have steadily increased over the last decade. Within the United States, the most frequently encountered category of NPS has become synthetic opioids. Increased overdoses resulting from consumption of these new compounds has contributed to a national epidemic, with devastating public health impacts. Due to the rapid and unpredictable changes in availability of substances, similarity of chemical structures, specialized instrumentation required to detect sub-nanogram concentrations, and lack of reference standards, the prevalence of these substances is undoubtedly underreported in forensic casework.

Objectives: The objective of this presentation is to describe the analysis of previously acquired high resolution mass spectrometry (HRMS) data in order to retrospectively identify novel opioids including fentanyl analogs that were not included in the original scope of testing. The goals of this project included evaluating the time between when these NPS were being sold on the illicit market and when they were first identified in toxicology casework, as well as determining trends in prevalence.

Methods: Deidentified HRMS data files were archived from postmortem and DUID cases from our collaborating laboratory (NMS Labs, Horsham PA) between 2018 and 2019. The datafiles were retrospectively re-processed against frequently updated databases with new and emerging compounds. All analytical data was previously acquired on an Agilent 1290 liquid chromatograph coupled to an Agilent Jet Stream 6230 time of flight mass spectrometer (LC-TOF-MS). Agilent MassHunter Qualitative Analysis software was used for the identification of compounds. Standard reference materials were analyzed on the same platform to generate retention time and accurate mass data that was used for processing. The re-processing library contained over 170 different opioids and/or fentanyl analogs. Presumptive positive results were flagged using the following criteria: retention time ± 0.100 minutes relative to the library, the ppm error associated with the parent mass of less than 20, acceptable chromatography, the presence of correct isotopic pattern, and overall score. For positive findings of new substances that were not part of the original scope of testing, the data archive was retrospectively mined to determine date of first appearance.

Results: The preliminary results from January to December 2018 demonstrated a total of 12 new opioids or fentanyl analogs identified through this process (Table 1). Benzylfentanyl was identified a total of 9 times during this time period; however, it was identified by retrospective datamining in 3 cases before its inclusion in the out of scope findings in June 2018. Isopropyl U-47700 was identified in a total of 10 cases and was identified for the first time in May 2018 by the CFSRE. Retrospective datamining identified a further five cases between March and April 2018; however, it has not been detected again since October 2018. Phenylbenzylfentanyl was identified using datamining on two different occasions, once in February and one in June 2018.

Table 1. Datamining results for data acquired between January and December 2018

Analyte name	# Identified	Date of 1st detection
Isopropyl U-47700	10	Mar-18
Benzylfentanyl (R-4129)	9	Jan-18
Benzylfuranylfentanyl	9	May-18
Phenylfentanyl	5	Jan-18
3,4-Methylenedioxy U-47700	3	Jan-18
Alpha'-Hydroxyacetylfentanyl	2	Aug-18
Alpha-methylbutyrylfentanyl*	2	Jun-18
N-methylnorfentanyl	2	Sep-18
Ortho/Meta/Para-fluorofuranylfentanyl	2	Dec-18
Phenylbenzylfentanyl	2	Feb-18
4'/Para-Methylfentanyl	1	Apr-18
Despropionyl-ortho/3-methylfentanyl	1	Aug-18

Conclusion/Discussion: The data shows that retrospective datamining can be a valuable tool to determine the prevalence and date of first appearance of novel compounds not contained within the initial scope of testing, without the need for re-extraction or retesting of the sample. Using datamining can help to evaluate the lag time between first detection and the incorporation of an analyte into the scope of testing to provide laboratories with an additional resource about how often scope updates are needed.

Keywords: LC-TOF-MS, Datamining, Novel Opioids

S44: Victims of the Consumer-driven Demands for the Cannabis and Hemp Industries – Case Studies of Poisonings

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Background/Introduction: Electronic cigarettes (e-cigs) were developed as a method for nicotine delivery by the aerosolization of a liquid formulation made of a ratio of propylene glycol and vegetable glycerin and/or a pharmaceutical and/or herbal remedy plus, potentially, a flavoring agent. When the electronic cigarette is activated, the e-liquid is vaporized, followed by rapid condensation into an aerosol that the user inhales. E-cigs have been adopted by users to also inhale drugs other than nicotine (DOTNs), including cannabidiol (CBD). CBD is a significant active ingredient of *C. sativa* and *C. indica.* It has been purported to have anti-convulsant, anti-nociceptive, and anti-psychotic properties and is a popular ingredient in e-liquids. A single CBD formulation has been approved by the United States Food and Drug Administration for the treatment of two rare forms of epilepsy. The Farm Bill recently legalized hemp production in the United States, from which CBD can be extracted. If hemp contains more than 0.3% THC, the plant is considered a non-hemp product. A federally unregulated consumer-driven market of cannabinoid products, in combination with the loosely regulated e-cig market, has created public health and public safety challenges with toxicological emergencies.

Objectives: The purpose of this research was to identify unlabeled and unexpected psychoactive compounds in CBD e-liquids submitted by persons claiming unexpected untoward effects.

Methods: Eight samples were received from individuals across the United States who had purchased CBD e-liquid products from either online or retail outlets for therapeutic purposes. All samples were extracted into methanol and analyzed using a Shimadzu QP2020 GC-MS. In brief, an aliquot was injected onto a DB-5MS capillary column. The GC oven was programmed to an initial temperature of 70 ºC followed by a 15 ºC/min ramp to a final temperature of 300 ºC held for 7 min with a total run time of 27 min. Analytes were identified using the SWGDRUG library and confirmed by comparing retention times and full scans using certified reference materials, where available.

Results: Seven of the eight samples contained CBD. The eighth sample contained MMB-FUBICA. The other 7 samples contained 5F-ADB, dextromethorphan, AMB-FUBINACA, or other cannabimimetic related compounds, including the JWH series. Other hemp related compounds were also identified.

Conclusion/Discussion: Most accounts were made by persons who claimed CBD use was for therapeutic benefit as opposed to recreational use. One account was described as having become addicted to CBD from recreational use. Other reports led to statements such as "I have not been able to leave my apartment for four days", "What happened next I can only describe as the situation rapidly devolving into the scariest night of my life – I felt like I was dissociating from reality", "one of the times I confronted [my son] in his room pale and glassy eyed", and "a 79 year old grandmother just wanted to be pain-free, but she had severe hallucinations". All persons were surprised by the effects but did not know what action could be taken to report the e-liquid companies or distributors. Several were concerned that any attention would have adverse recourse, both personally and professionally.

Analysis of the eight samples revealed a number of unexpected psychoactive compounds that accounted for the untoward effects the individuals described. The federally unregulated cannabinoid market, combined with an e-cig market with loose quality assurance requirements, has created a significant public health and public safety problem in the United States. As such, these cases highlight the need for the forensic toxicology community to publish findings from casework which could demonstrate the breadth of the problem and provide a warning with wider implications for public safety.

S45: Syncan or Syncan't, There is No Try

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Background/Introduction: Over the past ten years, forensic toxicologists have been battling the emergence of synthetic cannabinoids from the original JWH series, to AM variants, from indoles to INACAs. This year, compounds incorporating facets from each of these groups were identified in human performance casework. A common feature among these cases involves vaporized products, particularly those disguised within the seemingly innocuous trend of cannabidiol (CBD) use.

Objectives: This presentation raises awareness about prominent synthetic cannabinoid derivatives in forensic casework this past year.

Methods: Blood and/or urine specimens were submitted to the Division of Forensic Toxicology as part of suspected driving while intoxicated or military fit-for-duty investigations. Routine screening encompassed a drugs of abuse immunoassay for 9 classes of drugs along with an alcohol screen by headspace gas chromatography. If case history indicated potential CBD use, a CBD-specific liquid chromatography tandem mass spectrometry (LC-MS/MS) method was utilized as a sensitive and specific screening technique. If screens were negative, testing escalated to include liquid chromatography time of flight mass spectrometry (LC-QTOF/MS) with 5F-ADB, MMB-FUBICA, 5F-MDMB-PICA, AB-FUBINACA, FUB-AMB, along with their respective acid metabolites included within the library. Presumptive screens were confirmed by LC-MS/MS analysis, with a limit of detection at 0.1 ng/mL.

Results: Since late March 2018, our laboratory has identified parent drug along with metabolites in approximately 100 cases containing either single or multiple cannabinoids. In one incident, a 21-year-old male seemed to be behaving erratically. On multiple occasions, he had slurred speech and vomited. Confirmatory analysis of the urine specimen revealed the presence of 4F-MDMB-BUTINACA 3,3-dimethylbutanoic acid.

A second incident involved a young male who missed morning formation. When others went to check on him, he was found to be sluggish and his room smelled like a scented vapor. AB-FUBINACA and FUB-AMB 3-methylbutanoic acid (AB-FUBINACA metabolite 3) were confirmed in urine.

A third service member admitted to smoking CBD oil from a vape shop. The CBD analysis was negative, but FUB-AMB 3-methylbutanoic acid was confirmed instead. His companion also had FUB-AMB 3-methylbutanoic acid in urine, but 4F-MDMB-BUTINACA 3,3-dimethylbutanoic acid was also present.

In another case, security forces encountered a young male who was acting strangely and under the influence of an unknown substance. It was his third instance in the last three days where he was incoherent and lethargic. Alcohol, immunoassay, and CBD were negative, but 4F-MDMB-BUTINACA 3,3-dimethylbutanoic acid was subsequently confirmed by LC-MS/MS.

Conclusion/Discussion: Although it is difficult for most forensic laboratories to keep up with the changing landscape of designer drugs, case history and time of flight mass spectrometry is vital to identifying other potential substances that may be otherwise overlooked. In addition, CBD vaping liquids were previously described in scientific literature to contain synthetic cannabinoids, as well as other substances with potential impairing effects. These results demonstrate how important it is to stay abreast of current trends, frequently update methods with relevant synthetic cannabinoid compounds, and conduct synthetic cannabinoid analyses as part of routine forensic toxicology casework.

PLATFORM

S46:Quantification of NPS Stimulants in Post-mortem Blood without Using Reference Standards – Synergy of Nitrogen Chemiluminescense Detection and HRMS in Tentative Characterization of NPS

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Introduction & objectives: The appearance of new psychoactive substances (NPS) in the illicit drug market poses demanding challenges to the healthcare, law enforcement, and forensic toxicology laboratories. Focusing on the latter, we have worked on a method that is designed to tackle a common analytical problem related to NPS—the unavailability of authentic reference standards.

Methods: We introduce a new analytical platform in NPS bioanalysis which consists of gas chromatography (GC) coupled to a nitrogen chemiluminescence detector (NCD) and quadrupole time-of-flight mass spectrometer (QTOFMS), interfaced with atmospheric pressure chemical ionization (APCI) source. A two-way splitter directs the GC flow simultaneously to APCI/QTOFMS for tentative identification and to NCD for universal quantification. Chromatographic peaks observed in both detectors are aligned to elucidate the identity of the quantified peaks in the NCD chromatogram.

The universal quantification by NCD is based on the linear and equimolar response to nitrogen. Therefore, all nitrogen-containing drugs (approximately 90% of all drugs) are within the scope of this method. In our previous studies, we have concluded that GC coupling provides better sensitivity than liquid chromatography in quantitative NCD measurements, which is desirable in bioanalysis. To control quantitative analysis, three external calibrators were selected to cover for *prim*, *sec* and *tert*- amines (amphetamine, methamphetamine and MDPV, respectively).

For identification, we selected APCI as an ion source for GC-QTOFMS since the preservation of the protonated precursor ion is required for accurate mass-based tentative identification (± 2 mDa threshold). Using a data independent acquisition mode in GC-APCI-QTOFMS, the precursor and qualifier ion data were acquired without any preselection of the target ions.

We applied the GC-NCD-APCI-QTOFMS platform to study 38 illicit psychostimulants, with emphasis on NPS, in spiked post-mortem blood. The post-mortem blood samples were extracted with butyl chloride/ethyl acetate (3:1 v/v) at a basic pH followed by acylation with trifluoroacetic anhydride. In addition, eleven post-mortem blood samples obtained from routine casework were analyzed by the GC-NCD-APCI/QTOFMS method and the results were compared with an established electron ionization GC-MS method with appropriate calibration.

Results: In total, 35 out of 38 spiked psychostimulants were successfully quantified with a limit of quantification (LOQ) of 0.05 mg/L. The between-day accuracy was 62.3 – 143.3% (mean 93.5%, median 88.5%) and precision was 6.6 - 22.4% CV (mean 15.8%, median 16.1%). Unfortunately, we were unable to quantify dibutylone, MDBD and methylphenidate at low levels because a commonly occurring matrix components were obscuring the chromatographic peaks in the GC-NCD. The agreement between electron ionization GC-MS (with reference standards) and GC-NCD-APCI-QTOFMS (without reference standards) in eleven post-mortem blood samples containing 0.08 – 2.4 mg/L of amphetamine (n = 5), methamphetamine (n = 4) or MDMA (n = 4) was 62.3 – 117.3%.

Conclusion: This is the first study to apply the recently introduced GC-NCD-APCI/QTOFMS platform to the accurate mass-based tentative identification and quantitative estimation of drugs in human blood, simulating an analysis where no authentic reference standards were available. With this approach, identification and quantification can be confirmed retrospectively for cases where the structure of the NPS was initially unknown. We conclude that, from the limited number of techniques available within analytical toxicology, GC-NCD-APCI/QTOFMS is among the most viable approaches to instantly estimate concentrations of stimulant NPS in blood.

PLATFORM

S47: Laboratory-Based Evaluation of the DrugWipe 5S Oral Fluid Test

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Background/Introduction: Oral fluid has become an increasingly popular biological sample for drug testing in the investigation of driving under the influence of drugs (DUID) cases. Performing oral fluid drug testing at the point of contact (POC) is an especially useful tool for producing timely investigative information and supporting officer suspicions about drug use immediately in the field. This approach may facilitate police investigations, provide actionable drug use information about suspects, and save time and resources in the field.

Objectives: The objective of this research was to evaluate the performance of the Securtec DrugWipe® S 5-Panel against its claimed scope and analytical sensitivity. Specifically, the evaluation was designed to: 1) assess the device performance relative to the manufacturer's published cutoffs; 2) assess the ability to produce positive results in polydrug cases; 3) investigate the cross-reactivity of commonly encountered drugs, and metabolites and; 4) evaluate the effects of potential interferences (e.g. oral hygiene products, beverages and tobacco) on the device performance.

Methods: All testing was performed using authentic drug free, pooled oral fluid (30μL). Cutoff evaluations were performed at the cutoff (Table 1) and ±50% of the cutoff concentration in replicates of ten. The second part of the cutoff evaluation consisted of running a series of mixed drug controls containing the target analytes at various concentrations. Cross-reactivity was assessed for in triplicate for 24 commonly encountered drug metabolites, therapeutic drugs and other drugs known to cross-react on immuno-assay tests and miscellaneous other drugs at a concentration of 1000 ng/mL. Commonly encountered potential interferents (e.g. coffee, mouthwash, alcohol, tobacco) were assessed in a series of experiments to assess matrix effects and signal suppression. All results were recorded using an instrumented reader (WipeAlyser®). Results were scored as true positives (TP) if the analyte was present in the aliquot and detected by the device, irrespective of its concentration. With all negative results, the concentration of each drug in the aliquot was compared to the manufacturer's specified cutoff concentration. Results were processed using receiver operator characteristics (ROC) analysis.

Table 1.

Drug Assay	DrugWipe 5S Cutoff (ng/mL)
THC	5
Cocaine	10
Amphetamine	80†
Methamphetamine	80†
Opiates (morphine)	10

†DrugWipe has a combined amphetamine/methamphetamine panel.

Cutoffs reflect parent drug only.

Results: The overall sensitivity for the cutoff evaluations was 100% for THC, cocaine, amphetamine, and methamphetamine and 80% for opiates. The WipeAlyser® detected seven THC positive results, five morphine positive results, seven amphetamine and eight methamphetamine positive results at 50% below the cutoff concentration. With respect to cross-reactivity, the WipeAlyser® detected both MDMA and MDA at 100 ng/mL, benzoylecgonine at 500 ng/mL, codeine at 10 ng/mL, both hydromorphone and hydrocodone at 100 ng/mL, 11-nor-delta-9-THC-COOH was detected at 10 ng/mL and cannabinol at 100 ng/mL. The consumption of gum immediately prior to the test produced two false negative results for opiates, while tobacco consumption yielded four false positives; two on the amphetamines panel and two on the opiates.

Conclusion/Discussion: The DrugWipe 5S® has a relevant test panel, testing for the most commonly encountered illicit drugs in drivers: THC, amphetamine/methamphetamine, cocaine and opiates The WipeAlyser® detected seven THC positive results, five morphine positive results, seven amphetamine and eight methamphetamine at 50% below the cutoff concentration. Recommendations related to device performance specifications have been previously described in the ROSITA and DRUID projects. The ROSITA project recommended greater than 95% accuracy compared to 80% recommended by the DRUID project. When evaluating the cutoff results, the Drug Wipe 5S meets both sets of accuracy recommendations for all assays when using the WipeAlyser® analyzer. No false positive results were observed during the testing cutoff, mixed drug or cross-reactivity assessments.

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Background/Introduction: Monitoring drugs of abuse in the pain management population is critical due to their strong addictive potential and interference with narcotic treatment of pain patients, which can lead to potential discontinuation of the treatment. Physicians determine patient medication compliance by ordering individualized urine drug testing (UDT). Patient specific UDT order becomes a challenge when physicians need to decide whom to test and what tests should be ordered. Ordering illicit drug tests in conjunction with justification of medical necessity is not always a straightforward decision because patient's age, history of relationship with physician, and risk assessment are some of the factors that play a role in ordering UDT. It is a common belief that middle-aged to older population is less predisposed to the use of illicit drugs, and therefore not tested for their presence.

Objectives: To evaluate the prevalence of illicit drug use within the pain management population. Understanding the extent of illicit drug use in the middle-aged to older population to assist physicians in justifying the needs of ordering illicit drug tests as part of compliance, medical necessity and therapeutic monitoring.

Methods: National Spine and Pain Centers, LLC (NSPC) conducted a retrospective analysis of both prescription and illicit urine drug test results from 2014 to 2018. Tests were ordered for patients by pain management healthcare providers in order to determine compliance and therapeutic monitoring. All positive illicit drug results were reviewed and classified into groups based on age and sex of the patients. Heroin and cocaine were determined based on the presence of their metabolites 6-acetylmorphine and benzoylecgonine, respectively. Ecstasy and PCP were identified by parent drug presence. Illicit D-methamphetamine isomer was distinguished from L-methamphetamine isomer (from Vicks Vapor Inhaler), while amphetamine was included if it was not prescribed.

Results: This study included review of 300,000 UDT reports for patients of 45 years and older (male (41%)/ female (59%)), which would represent 82.3% percent of all pain population in NSPC clinics.

Age group	Heroin metabolite (6-AM)	Cocaine metabolite (Benzoylecgonine)	Ecstasy (MDMA/ MDA)	Methamphetamine	PCP
45-54	0.50%	3.71%	0.01%	0.15%	0.13%
55-64	0.30%	2.50%	0.00%	0.12%	0.10%
65-74	0.12%	0.98%	0.00%	0.02%	0.02%
Over 75	0.00%	0.35%	0.00%	0.00%	0.02%

Cocaine was found to be the most prevalent drug of abuse in all groups of middle age to older population. Ecstasy was the rarely used in middle-aged to older population. Heroin, cocaine and PCP were about two times more prevalent in males, while amphetamine was about two times more prevalent in females. Methamphetamine was equivalently found in both sex groups. Our research found the majority of the drugs of abuse were found in conjunction with prescribed medications – opioids, benzodiazepines, skeletal muscle relaxants, or even with several combinations of illicit drugs. Amphetamine and cocaine, cocaine and heroin (Speedball) are the top illicit drug combinations.

Conclusion/Discussion: Cocaine can be recommended for testing more frequently in middle-aged to older population, while ecstasy is rare in the same age group. The pain population is very vulnerable to abusing illicit drugs and their use is often underestimated and underdiagnosed. This study and future studies will help physicians to justify their UDT orders of illicit drugs in pain management practices.

PLATFORM

S49: Implementation of a Blind Quality Control Program in Blood Alcohol Analysis

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Background/Introduction: Declared proficiency tests (PTs) are beneficial to laboratories to measure results and processes against other laboratories in the country but are typically completed once per year per analyst as part of the accreditation requirement. Declared PTs are limited in their use for testing the performance of the entire system because analysts are aware that they are being tested. A blind quality control (BQC) sample is intended to appear as a real case to the analyst to remove any intentional or subconscious bias. By removing bias, laboratories can more effectively and realistically gauge the performance of their processes.

Objectives: To provide continuous quality improvement and a constant and real-time assessment of the laboratory processes, the Houston Forensic Science Center (HFSC) incorporated a BQC program in blood alcohol analysis in September 2015 as a supplement to declared PT tests. It allows HFSC to monitor the reliability of procedures used in casework from evidence intake to reporting.

Methods: Between September 2015 and July 2018, HFSC submitted 317 blind cases: 89 negative samples and 228 positive samples at five target concentrations (0.08, 0.15, 0.16, 0.20, and 0.25 g/100 mL; theoretical targets). For the blind samples to mimic real toxicology cases, the case information and its associated paperwork were fabricated and placed, along with the blood tubes, into typical collection kits used by the submitting agency. These BQC cases were submitted, received, and analyzed by HFSC in the same manner as real evidence. These samples were analyzed by a headspace gas chromatograph interfaced with dual flame ionization detectors (HS-GC-FID); the limit of quantification for ethanol was 0.01 g/100 mL.

Results: All negative samples produced "no ethanol detected" results. The mean (range) of reported blood alcohol concentrations (BAC) for the aforementioned target concentrations was 0.075 (0.073-0.078), 0.144 (0.140-0.148), 0.157 (0.155-0.160), 0.195 (0.192-0.200), and 0.249 (0.242-0.258) g/100 mL, respectively. The average BAC percent differences from the target for the positive blind cases ranged -0.4 to -6.3%, within our uncertainty of measurement (8.95-9.18%). The rate of alcohol evaporation/degradation was determined negligible. A multiple linear regression analysis was performed to compare the % difference in BAC among 5 target concentrations, 8 analysts, 3 HS-GC-FID instruments, and 2 pipettes. The variables other than target concentrations showed no significant difference (p-values >0.2). While the 0.08 g/100 mL target showed a significantly larger % difference than higher target concentrations (0.15-0.25 g/100 mL), the % differences among the higher targets were not concentration-dependent. This suggested that the source of the theoretical target level variability was likely from the manufacturing process rather than the systemic bias from the analytical method.

Conclusion/Discussion: Despite difficulties like gaining buy-in from stakeholders and mimicking evidence samples, the implementation of a BQC program has improved processes, shown methods are reliable, and added confidence to staff's testimony in court. The 2017 revision of the General requirements for the competence of testing and calibration laboratories standard published by the International Organization for Standardization/International Electrotechnical Commission (ISO/IEC 17025) requires laboratories to monitor the validity of their results. Clause 7.7.1 lists out several quality controls that a laboratory may implement to conform to this requirement, and the BQC program fulfills both 7.7.1 j) intralaboratory comparisons and 7.7.1 k) the testing of blind samples. HFSC has yet to receive an unsatisfactory result from a blood alcohol analysis BQC case. The results indicate no significant variation in blood alcohol results over time, between analyst, instrument, or pipette. Therefore, we can conclude that our methods are reliable and produce accurate results. HFSC encourages other forensic laboratories to consider implementing their own BQC program that is tailored to their specific needs as a reliable means for evaluating their processes and complying with accreditation requirements.

PLATFORM

S50: Endogenous GHB in Hair: A Large Population Study

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Background/Introduction: Drug-facilitated crimes (DFC) can be difficult to corroborate with traditional toxicology samples like blood or urine due to reporting delays. Hair can often be a useful sample addition for testing in DFC cases as it provides a longer detection window (1-3). Delay in reporting a DFC that involves GHB (gamma-hydroxybutyric acid) is also complicated by its rapid metabolism and clearance from the body, as well as its endogenous presence. While many studies over the years have worked to resolve the challenges encountered when analyzing GHB in hair, few have had large populations. Additionally, new statistical approaches for demographic hair comparison and differentiation of endogenous and exogenous GHB are discussed.

Objectives: By testing a large population of non-GHB users, the audience will be able to understand and draw conclusions about endogenous GHB concentrations with the goal to better differentiate endogenous from exogenous GHB in hair.

Methods: Hair samples and demographic information were collected with documented informed consent from 214 donors. The hair samples were cut close to the scalp, segmented into 1 cm portions, decontaminated, and ground to a fine powder. A 10 mg sample of each segment was processed by 1 M NaOH base digestion, ethyl acetate liquid-liquid extraction, and liquid chromatography tandem mass spectrometry analysis. An Acclaim® Trinity™ P1 column (2.1 mm × 150 mm, 3 μm) with guard column (2.1 mm x 10 mm, 3 μm) was used. Mobile phase A contained 45% 25 mM ammonium acetate at pH 5.49 and 55% acetonitrile; mobile phase B was 100% water (Optima LC-MS grade).

Estimations of GHB in the donor samples were completed using a calibration curve prepared by spiking GHB into SMx™ Hair liquid matrix (UTAK®, Valencia, CA) from 0.4-12.0 ng/mg. The quantitative data were analyzed using Minitab™ and Excel™ software packages along with bootstrapping and Kruskal-Wallis statistical tests.

Results: A total of 2074 hair sample segments from 141 women and 73 men (all collected hair 3-12 cm in length) were analyzed. The range of endogenous GHB concentrations observed was <0.4-5.5 ng/mg and 97.5% of the segmental results were less than 2 ng/mg. Statistical analysis was performed on a segmental basis and the results (e.g., median, etc.) will be discussed. A Kruskal-Wallis comparison of segmental medians in males and females indicates that these groups are different, with greater than 95% confidence. Samples from 73 female donors in this study experienced some type of chemical and/or thermal treatment within the year prior to collection. Comparing "treated" to "untreated" hair in the female group led to subpopulation differences, with greater than 95% confidence. Age groups and races were also analyzed for differences, but none were significant at $\alpha=0.05$.

Female hair samples appeared to have a trend comprising higher endogenous GHB concentrations close to the scalp and with a net decrease of ~0.2-0.3 ng/mg distally. Male hair samples displayed the opposite trend, with a net increase of ~0.5-0.6 ng/mg from the proximal to the distal end of the hair shaft. However, there was minimal change between individual adjacent hair segments, with 97.1% of adjacent segment differences within ±0.5 ng/mg, across the population.

Conclusion/Discussion: This is the largest hair population of non-GHB users studied to date. The endogenous GHB concentration range was largely consistent with previously published data (4). Based on the wide concentration range detected in our population, it appears difficult to select an appropriate cut-off for differentiating endogenous GHB from exogenous without a large controlled dosing study. There was differentiation based on gender and chemical/thermal treatment. Other variables did not significantly differentiate subpopulations. Additionally, using adjacent segment concentration differences could be a strategy to assist in differentiating endogenous from exogenous GHB exposure.

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PLATFORM

S51: Interest and Interpretation of Hair Analysis in Mistreatment of Hospitalized Elderly People

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Background/Introduction: In France, 600,000 elderly people are in accommodation facilities for dependent people and more than half have cognitive disorders or dementia.

Objectives: To identify the cause of the disappearance of some psychotropic drugs in the pharmacy of a Geriatric Long-Term Care Unit.

Methods: Many cases of missing drugs were reported by the executive nurse in a geriatric Long-Term Care unit. Different psychoactive drugs were implicated such as midazolam while no patients had such a prescription. Four hypotheses have been proposed: 1. stolen by a nurse for his own addiction, 2. stolen by a nurse in order to resale, 3. mistaken in traceability of inventory movements of the drugs, and 4. misused of the drugs without prescription by a nurse to calm the restless patients during the night. In order to exclude mistreatment, blood and hair samples were successively analyzed without the knowledge of the staff early in the morning for 5 patients with the most frequent agitation crises. When hair was sampled, a 2-cm segmental analysis was performed when possible (length ≥3cm). TSQ Endura LC/MS/MS targeted screening and quantitation (Thermo Fisher®) were performed in blood or a 20 mg-hair sample after decontamination and Liquid/Liquid extraction in basic conditions. The method of quantification was accredited and included all psychoactive drugs such as drugs-of-abuse, neuroleptics, hypnotics, antidepressants, anxiolytics, anti-epileptics, and other sedatives. Limits of quantification were between 1 and 10 ng/mL or pg/mg according to the compounds.

Results: In the first blood analysis, one of the patients was found positive for midazolam (0.9 ng/mL) and OH-midazolam (0.6 ng/mL). This patient initially tested negative with a TSQ Access MAX triple quadrupole method (Thermo Fisher®), a less sensitive instrument compared to the TSQ Endura, showing that instrument sensitivity is critical in suspected mistreatment cases. This result indicated the administration of a non-prescribed compound in this patient. Hair testing performed in 5 additional patients showed the presence of non-prescribed midazolam. The concentrations were low in 4 of the patients (1-12 pg/mg) in accordance with single administration and was found higher in the last patient (40-120 pg/mg in the different segments). Additional non-prescribed therapeutic drugs were also identified in these 5 patients' hair: zolpidem (4 patients), carbamazepine (4), clobazam (3), tramadol (2), nefopam (1), lamotrigine (1), zopiclone (1), alprazolam (1), levetiracetam (1), citalopram (1), venlafaxine (1), and codeine (1). Each patient tested positive for 2 to 9 non-prescribed compounds in their hair. After implementing modified security and delivery of the pharmaceuticals in the unit, blood samples were analyzed 3 months later. These results revealed that 3 patients were still positive for a non-prescribed medicine, 1 for zopiclone (while this substance was no longer available in the unit), 1 for clobazam (not available), and 1 for paroxetine (still available for the nurse staff). The corresponding hair analysis performed simultaneously on the 5 same patients showed the disappearance of all the benzodiazepines and "Z drugs" (midazolam, alprazolam, zolpidem, zopiclone) except clobazam still present in 2 patients (concentrations decreased relative to initial test results). Carbamazepine (4), lamotrigine (1), and levetiracetam (1) were still present and two new antidepressants appeared in patients at low, possible single dose levels, mirtazapine (2) and venlafaxine (1). These low positives could be due to a mistakenly and unintentionally administration since different patients in the same room received these treatments as those that erroneously tested positive. After reporting to the prosecutor, a press conference by the General Manager of the "Assistance Publique - Hôpitaux de Paris" led to the secure delivery of all psychotropic drugs in all the geriatric departments of our institution and suspension of a nurse suspected of improperly administering medications. In order to confirm the disappearance of mistreatment, 19 patients' hair including the 5 initial patients were collected 6 months later and confirmed the absence of non-prescribed psychoactive substances in the 1 or 2-cm proximal hair segment.

Conclusion/Discussion: This study shows the need for a sensitive toxicological analysis and the reliability of hair testing in suspected mistreatment cases of hospitalized elderly people. Interpretation of results from blood and hair needs to be delicately considered as there is the possibility of accidental drug administration mistakes due to multiple drugs for patients in shared rooms.

PLATFORM

S52: Toxicological Findings in Victims of Sexual Assaults with and without the Suspicion of Drug Facilitated Crime (DFC)

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Background/Introduction: It is not unusual that a victim suspects they were drugged in connection with a sexual assault or other violent crime. However, earlier studies from our laboratory have shown that the presence of other substances than ethanol in victims is scarce and concluded that drug facilitated sexual assaults are rare. The fact that more than half of the victims had ethanol on board pointed towards that as the primary cause of impairment. During 2011, the National Board of Forensic Medicine introduced a routine drug and medication screening using high resolution mass spectrometry in all violent crimes, increasing the chances to detect substances commonly used in drug facilitated crimes.

Objectives: The aims of our study were to describe the toxicological findings in cases of violent crimes and compare the results from cases where the victim reported a suspected drugging or when they did not.

Methods: Retrospectively, we retrieved information from the National Board of Forensic Medicine's case database. All cases from victims of suspected violent crimes between 2012 and 2018 were identified. Cases were the suspected offense included "rape", "sexual assault", "spiking", or "drugging" were then selected. Based on the police request the cases were divided into two groups, no suspected drugging and DFC.

Results: There was a total of 3903 cases of violent crime where 2813 were classified as sexual assaults or druggings. Because of lack of background data 344 could not be grouped resulting in a study population consisting of 1100 DFC cases and 1369 cases where no drugging was suspected.

The mean age was 25.0 and 25.4 in the DFC and non-DFC groups. In 32.8 % of the DFC cases the toxicological analyses were completely negative as compared to 30.3 % in the non-DFC group. Ethanol detection rate was significantly different between the two groups with 48% of the cases with no suspicion of drugging and 41% in the DFC group. The mean blood alcohol concentration was 1.14 promille (0.12 g/dL) and 1.06 promille (0.11 g/dL), respectively with no significant difference between the two groups. In DFC cases 17% had one or more hypnotic or sedative drug positive whereas in the other group only 11% were positive suggesting an overrepresentation in DFC cases. The same trend was seen for central stimulants (12.4% vs 7.5 %), opioids (7.7% vs 5.8%), and cannabis (10.3% vs 6.6%). Other medications, on the other hand, was more prevalent in the non-DFC group with 23.5% positives compared to 12.3%. The most common sedatives found in blood were diazepam, alprazolam, and clonazepam. The concentrations were within the therapeutic ranges and did not differ between groups. However, therapeutic concentrations do not preclude impairment, especially not in naive users or when combined with alcohol.

Conclusion/Discussion: In conclusion, there were significant differences in findings between DFC cases and cases where no drugging was suspected. We found a lower proportion of ethanol in the DFC group but the mean BAC was similar. DFC cases had a higher proportion of abused drugs and sedatives present. Indeed, one out of six of the DFC cases had a sedative on board pointing towards a potential drugging.

PLATFORM

P01: Evaluation of Acetylfentanyl following Suspected Heroin Overdose when Complicated by the Presence of Toxic Fentanyl and Alprazolam concentrations

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Background/Introduction: A 34-year-old male was reported to be snorting a white powder that was initially believed to contain heroin. Toxicological analysis revealed morphine, fentanyl with trace norfentanyl detected only in stomach contents, alprazolam, and acetylfentanyl in femoral blood, and 6-monoacetylmorphine (6-MAM) in vitreous fluid. The fentanyl and alprazolam concentrations may normally be associated with a fatal outcome, and this is supported with the distribution of fentanyl and alprazolam being consistent with an acute intoxication. In addition, the presence of 6-MAM and a free versus total morphine ratio of 67.9% provide supporting evidence of a rapid death. However, the presence of illicit acetylfentanyl complicates toxicologic interpretation due to overlapping recreational and fatal concentrations of this compound reported thus far in the literature as well as a potential for postmortem redistribution. Acetylfentanyl concentrations reported in the literature have also varied when presented with significant fentanyl concentrations, and underscore the need to consider a wide range of illicit opioid compounds when investigating apparent drug-related deaths. Based on our toxicologic analysis, the results suggest an acute intoxication primarily by acetylfentanyl and fentanyl. In addition, we suggest that the presence of alprazolam, 6-MAM, and a high % free morphine are also consistent with a rapid death. The cause of death was officially attributed to an acute combined intoxication of acetylfentanyl, fentanyl, alprazolam, and heroin, with the manner of death as accidental.

Objectives: Presenting a postmortem case where the interpretation of acetylfentanyl, fentanyl, and alprazolam concentrations is described following toxicologic analysis.

Methods: Acetylfentanyl, fentanyl, and norfentanyl were isolated from case specimens by means of an alkaline liquid/liquid extraction scheme. In short, an aliquot of the specimen is mixed with a strong base to convert the drugs to a non-ionized state. A volume of non-polar solvent is added into which the non-ionized drug will transfer. The resultant solvent layer is evaporated to dryness, reconstituted in mobile phase, and analyzed by LC–MS-MS. Acetylfentanyl was quantified by LC–MS-MS using a four-point calibration curve. Fentanyl and norfentanyl were quantified by LC–MS-MS using a five-point calibration curve. Alprazolam was quantified using solid-phase extraction followed by LC–MS analysis using a four-point calibration curve. 6-MAM and free morphine were also quantified using SPE with oxime and trimethylsilyl derivatization followed by GC–MS analysis. Both were calibrated using five-point calibration curves. For total morphine, enzyme hydrolysis with β-glucuronidase in a water bath at 65°C preceded SPE. Complete hydrolysis was confirmed using a morphine-3-glucuronide control. All quantitative methods incorporated the use of deuterated internal standards and matrix-matched controls to ensure proper calibration.

Results:

Table I. Case Distribution Results

Drug	Femoral blood (µg/L)	Cardiac blood (µg/L)	Brain (µg/kg)	Liver (µg/kg)	Stomach contents (µg/total)	Vitreous (µg/L)	Urine (µg/L)
Fentanyl	34.7	36.1	54.5	52.4	2.20	NA[a]	NA
Norfentanyl	ND	ND	ND	ND	<1.00	NA	NA
Alprazolam	64.9	61.1	105	208	1.18	NA	NA
Free morphine	356	285	153	519	13.4	QNS	NA
Total morphine	524	467	NA	NA	NA	NA	ND
6-MAM	NA	NA	NA	NA	NA	<10.0	ND
Acetylfentanyl	32.9	24.4	46.9	55.3	1.07	NA	NA

a. Abbreviations: NA, not analyzed, ND, not detected, and QNS, quantity not sufficient

Conclusion/Discussion: The results suggest an acute intoxication primarily by intranasal administration of acetylfentanyl and fentanyl. In addition, we suggest that the presence of alprazolam, 6-MAM, and a high % free morphine are consistent with a rapid death. The cause of death was officially attributed to an acute combined intoxication of acetylfentanyl, fentanyl, alprazolam, and heroin, with the manner of death as accidental. Prolonged abstinence and limited access to traditional drugs of abuse resulting from rehabilitation may have lead to the loss of opioid tolerance and subsequent misuse of alprazolam as well as what the decedent might have believed to be only heroin. The now common adulteration of heroin with fentanyl or fentanyl analogs like acetylfentanyl in addition to the wide availability of benzodiazepines such as alprazolam appears to have lead to a rapid demise.

POSTER

P02: Fatal Nitrous Oxide (N2O) Abuse: Toxicological Findings in a Putrefied Case

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Background/Introduction: Nitrous Oxide (N_2O) is a gas legally used in the medical field for its analgesic properties and in the food industry as a foaming agent for whipped cream. This volatile substance can also be abused for its euphoric and anxiolytic effects. The ease of access, low cost and short duration of psychoactive effects for this substance might contribute to a banalization of the potential harms and dangers incurred by recurrent abuse. Indeed, chronic nitrous oxide use has been reported to cause vitamin B12 deficiency leading to myelopathy with or without neuropathy. It has also been linked with homocysteine accumulation which increases risks of thrombocytosis. Ultimately, acute nitrous oxide abuse can be fatal due to the replacement of oxygen in the airflow, causing hypoxia which can escalade to asphyxia and death.

Objectives: We are reporting case history and complete toxicological findings in a case of fatal nitrous oxide abuse where N_2O confirmation results were obtained 29 days after the initial analysis, despite extensive sample manipulation during this period of time.

Methods: Biological matrices were collected at the mortuary and sent to the laboratory for toxicological analyses. Femoral blood, blood drawn from the jugular vein and vitreous humour were collected in glass tubes containing sodium fluoride and potassium oxalate (BD Vacutainer), whereas urine was collected in a plastic tube without preservative. Samples were stored at 4°C until completion of toxicological analyses. Targeted screening of 144 xenobiotics was achieved with protein precipitation extraction followed by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS, 5500 QTRAP®, Sciex). Detection and quantification of N_2O was performed via GC/MS by NMS Labs (Willow Grove, PA, USA).

Results: A 28-year-old male was found lying on his bed, with a plastic trash bag over his head tightened with a belt and a condom. He was last seen alive 3 days before being discovered by the police. External signs of putrefaction were apparent. No suicide note could be found, and the deceased had not displayed suicidal intent. Several whipped cream chargers and a professional whip cream dispenser were discovered on the scene, along with multiple condom boxes, sex toys and garbage bags. Cannabis was present on the scene along with an electronic cigarette. Routine toxicological analysis only revealed the presence of THC-COOH in urine (11-nor-9-carboxy-Δ9-tetrahydrocannabinol). Analysis of blood and vitreous humour samples were negative, including for ethanol. Both glycemia and carboxyhemoglobin were at normal levels. N_2O was first detected at the external laboratory in femoral blood (< 9 μg/mL) and in blood from the jugular vein (19 μg/mL) 30 and 38 days, respectively, after sample collection. Confirmation analysis on femoral blood was successfully carried out 29 days later after undergoing four cycles of sample reaching room temperature, tube opening, aliquoting and re-storing at 4°C. Nitrous oxide was not detected in urine.

Conclusion/Discussion: Nitrous oxide was detected in both peripheral and central blood. Quantification in femoral blood was not possible due to the method's limit of quantification. However, quantitative results for nitrous oxide are of limited relevance to the forensic toxicologist. Indeed, it is a volatile substance with unknown stability in the uncontrolled environment of the scene of death. Toxicity interpretation based on concentration is thus perilous. Furthermore, identification alone is sufficient to conclude to intake, since this compound has no endogenous presence, although ammonia is known to be processed to N_2O and N_2 during the anaerobic denitrification by bacteria in soil graves. This case also demonstrates that despite the volatile nature of nitrous oxide, detection can still be achieved in biological matrices even after a long storage period and extensive sample handling. Nonetheless, N_2O presence could be confirmed analytically via a dedicated method not carried out routinely. Thus, this case report is an eloquent demonstration of how important extensive death scene description is to a forensic toxicologist. Here, the peculiar observation of whipped cream material guided the appropriate testing and led to the confirmation of a probable cause of death.

P03: A Fatal Case of Methomyl Poisoning Identified via QTOF Screening

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Background/Introduction: Methomyl is a carbamate-based insecticide that inhibits cholinesterase activity, causing symptoms such as blurred vision, sweating, convulsions, nausea, and vomiting. Animal studies of acute methomyl exposure have shown it to be highly toxic orally and poisonings via inhalation and transdermal absorption have also been reported. We describe the fatal case of a 35-year-old woman who presented at the emergency room with sweating, convulsions, muscle weakness, eye twitching, and mouth numbness. She did not have a history of any illness and was not taking any medications. Despite a thorough workup at the hospital, no specific diagnosis was made, and she was discharged. Upon arriving home, her symptoms resumed. She died shortly thereafter. Prior to this, the decedent had stated that her husband should be investigated in the event of her death.

Objectives: This poster will serve to introduce members of the community to a unique case study in which an analyte of interest was not part of the LC-QTOF-MS/MS routine drug screen but was later identified using a retrospective non-targeted search.

Methods: Each sample consisted of a 200µL aliquot and dilutions were performed as applicable. 50µL of internal standard was then spiked into each tube. 800µL of acetonitrile was added dropwise while vortexing, after which the tubes were centrifuged for 10 minutes at 4400rpm. Supernatants were then transferred and evaporated to dryness. Samples were reconstituted with 200µL of 20% methanol:water and vortexed. Following reconstitution, tubes were centrifuged for 10 minutes at 4400rpm and transferred to autosampler vials for injection. The instrumentation used included a Shimadzu Nexera Liquid Chromatography System with a Restek Raptor Biphenyl 100x2.1mm 2.7µL HPLC column and a SCIEX X500$_R$ Mass Spectrometer. The ionization source was a Turbo V with Twin Sprayer ESI Probe. Chromatographic separation was achieved using a binary gradient of solvent (A) 0.1% formic acid in water and solvent (B) 0.1% formic acid in methanol at a flow rate of 0.6mL/min. Initial gradient conditions were 5% B for nine minutes, followed by one minute of 100% B, and finally 5% B for the remaining two minutes. The QTOF was operated in IDA mode using positive electrospray ionization. Fragmentation was achieved using a collision energy of 35V with a spread of ±15V. Data was initially processed using SCIEX OS Software 1.4 with an extracted ion chromatogram list containing over 300 analytes. A non-targeted approach can also be employed. An in-house library was created using CRMs to establish retention time and fragmentation criteria.

Results: A targeted drug screen via QTOF consisting of approximately 350 drugs did not reveal anything out of the ordinary. However, one of the benefits of using a QTOF screen is the ability to retrospectively analyze data which was previously generated, negating the need to resample and re-extract. At the investigating agency's request, a non-targeted approach was used to reprocess the data and methomyl was subsequently identified in the sample.

Conclusion/Discussion: The QTOF method described is currently used by the Dallas County Southwestern Institute of Forensic Sciences as part of a routine drug screen. This method allows for short analysis time with a large scope of analytes available in the library. The ability to retrospectively "data mine" in a non-targeted approach allows the laboratory to search for additional analytes that may become significant as more information in an investigation becomes available.

P04: Postmortem Distribution of N,N-Dipropyltryptamine in an Intoxication Case

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Background/Introduction: N,N-dipropyltryptamine (DPT) is a synthetic *N,N*-dialkylated tryptamine possessing strong affinity for the 5-HT$_{2A}$ receptor. DPT is unscheduled in the United States but may be considered an analog of structurally similar controlled substances. DPT is a Schedule I controlled substance in the states of Florida, Maine, and Oklahoma. DPT was briefly investigated as an adjunct to psychotherapy in the 1960s and 1970s. Intramuscular doses of 10 to 165 mg were administered to patients with no adverse physical effects. During these studies, DPT was observed to produce enhanced recall, deeper self-exploration and greater psychodynamic resolution. Little is known about the pharmacokinetics and pharmacodynamics of DPT in humans.

Case History: The death of an 18-year-old male was reported to the Office of the Chief Medical Examiner of Maryland. History indicated the decedent was last known alive by a friend. According to the friend, the two men walked into a wooded are with the intent of consuming mushrooms and spending the night in the woods. The friend told police that he consumed mushrooms while the decedent snorted a white powder that he purchased on the dark web. The decedent became unresponsive soon after insufflating the powder. Emergency assistance was summoned immediately. Despite resuscitative efforts, the man was pronounced deceased at the scene. Autopsy findings revealed the upper airway to be clear of debris and the pulmonary parenchyma was red-purple, exuding mild to moderate amounts of blood fluid. Other autopsy findings were unremarkable. Postmortem toxicology testing was positive for bupropion, citalopram and N,N-dipropyltryptamine.

Objective: There is limited literature describing N,N-dipropyltryptamine intoxication. This study presents the postmortem distribution of N,N-dipropyltryptamine from an intoxication death.

Methods: DPT was quantitated using the lab's alkaline drug extraction procedure. Briefly, internal standard (Mepivacaine) was added to specimens which were alkalinized and extracted with n-butyl chloride then back extracted into sulfuric acid and finally alkalinized and extracted into methylene chloride. Isopropanol was added, and the extract was evaporated to the isopropanol layer which was injected into the GC-NPD for analysis. The method was linear from 0.025 mg/L to 1.0 mg/L. A six-point calibration curve and three control concentrations were included in each batch. Specimens were analyzed at a dilution to produce results within the linear range.

Results:

Specimen	N,N-dipropyltryptamine	Other findings
Heart Blood	1.3 mg/L	Bupropion: 0.08 mg/L
		Citalopram: 0.5 mg//L
Femoral Blood	1.2 mg/L	
Urine	3.2 mg/L	Bupropion positive
		Citalopram positive
Liver	4.5 mg/kg	
Kidney	2.6 mg/kg	
Vitreous Humor	0.30 mg/L	
Bile	1.5 mg/L	
Gastric Contents	12 mg in 230 mL total	

Conclusion/Discussion: DPT was detected in all specimens analyzed, suggesting that multiple matrices are appropriate for detection of DPT use. There is little difference between central and peripheral blood concentrations and the liver to peripheral blood ratio is 3.7, suggesting that DPT may have a lower propensity for postmortem redistribution. The lower concentration in vitreous humor may be a result of the death occurring within a short time after use. There are no reported cases of DPT intoxication for comparison of blood concentrations.

Keywords: postmortem, N,N-dipropyltryptamine, intoxication

POSTER

P05: Suicide by…Suicide Tree? A Case Study and Analysis of *Cerbera odollam*

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Introduction: *Cerbera odollam* is a tree that grows natively in marsh and swamp areas of India and southeast Asia and goes by the common name "Pong-pong" and the more sinister moniker "Suicide Tree." Suicide by ingestion of the fruit's seed kernel is a common means of suicide, particularly in the Kerala state of India, where it grows wild and its lethality well-known. As little as one-half a kernel can bring about death.

The primary mechanism of toxicity is by the steroidal cardiac glycoside cerberin and related compounds such as neriifolin, tanghinin, and deacetyltanghinin. These cardiac glycosides bind to and inhibit cellular Na/K-ATPase, the so called "sodium/potassium pump," much like the more familiar digoxin and oleandrin.

While not indigenous to the United States, the dried seed can be easily purchased online, and the kernel ingested for suicide. In mid-2018 a case presented to the NC OCME toxicology laboratory of just such a case: A 28-year-old woman visiting from out-of-state, with a history of schizoaffective disorder and a recent suicide attempt with antifreeze, purchased "Pong-pong" seeds online and ingested them for the purpose of suicide.

Objective: To develop a high-resolution, accurate-mass screening method for cerberin, neriifolin, tanghinin, and deacetyltanghinin in order to help establish the means and manner of death in a postmortem medico-legal case.

Methods: A qualitative, multi-analyte targeted assay using a high-resolution, accurate-mass Thermo Orbitrap LC-MS/MS was developed. Analytes of interest were extracted from 0.1 mL of postmortem blood. At the time of analysis, cerberin and neriifolin were the only reference standards that could be easily obtained. The accurate masses of tanghinin and deacetyltanghinin were input into the method and full scan spectra compared to those observed in the literature.

Results: While the analysis of postmortem blood did not detect cerberin, it did reveal the presence of neriifolin, tanghinin, and deacetyl tanghinin, markers for *Cerbera odollam* ingestion. Because neriifolin was the only reference standard available for the three detected analytes, it was the only cardiac glycoside reported. Additional, standard drug toxicology detected only a trace amount of trazodone. In conjunction with autopsy and investigation findings, the case was signed out as a suicidal poisoning by *Cerbera odollam*.

Discussion: Suicide by *Cerbera odollam* is extremely rare in the United States but is frequently discussed in online suicide forums. Together, with being readily available to purchase online, suicidal ingestion of seeds from the "Suicide tree" could enter postmortem laboratory casework. In the case presented here, medical examiner investigation revealed that the decedent purchased seeds online, leading us to develop a successful method for the qualitative analysis of cerberin and other cardiac glycosides.

Keywords: *Cerbera odollam*, cerberin, neriifolin, postmortem

POSTER

P06: Utilization of Rapidfire-QTOF for Postmortem Blood Screening

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Background/Introduction: Current screening techniques utilized for postmortem blood via liquid chromatography time of flight mass spectrometry (LC-TOF-MS) often involve lengthy chromatographic methods that incorporate retention time for compound identification. Isobaric interferences and retention time shifts may produce falsely elevated counts and false positive results. In addition to long run times, large screening panels pose a challenge when trying to add additional analytes due to concerns over the impact to existing compounds. Agilent's Rapidfire 360 (RF) provides ultrafast injection time through a solid phase extraction (SPE)-like cartridge averaging less than 15 seconds per sample. When used in conjunction with a Quadrupole Time of Flight Mass Spectrometer (QTOF-MS), spectral fragmentation data can be generated that is matched to a Personal Compound Database Library (PCDL) for accurate determination of positivity in a sample.

Objectives: Feasibility studies were conducted utilizing Agilent's RF-QTOF as a qualitative screening technique for postmortem blood samples. Various modes of acquisition were compared to determine the optimal workflow for acquisition and data analysis.

Methods: Twenty-six analytes representing commonly identified drugs were fortified into 8 different human whole blood samples and extracted via a supported liquid extraction (SLE) prior to injection on the RF-QTOF. The RF method was developed to retain morphine with the shortest load time possible while utilizing a longer elution time to maximize points across the peak when utilizing auto-MS/MS. Samples were injected onto a reverse phase C18 cartridge utilizing 0.1% formic acid in water and eluted off of the cartridge with 0.1% formic acid in 90:10 methanol:water. The average injection time was 10 seconds sample to sample. A data dependent auto-MS/MS acquisition method was developed on the QTOF that would fragment precursors of interest at various collision energies when above a set abundance threshold. Fragmentation data was compared to a PCDL containing the analytes of interest. Database and library scoring was used to evaluate compound identification. Collision energies were optimized on an analyte by analyte basis.

Results: All of the 26 analytes were optimized to distinguish true positives from blank extracted human whole blood by adjusting collision energies used during fragmentation. The minimum database score was 89.67 and the minimum library score was 67.11 (the library score that resulted from an extracted blank blood for the same analyte was 11.14 for comparison). The current LC-TOF method used for postmortem screening required 10 minutes sample to sample while the RF-QTOF method developed required 10 seconds sample to sample which gave an approximately 60 fold increase in throughput of injected samples. A set of discarded samples (n=26) from the LC-TOF method were injected on the RF-QTOF with 121 analytes found positive via LC-TOF and RF-QTOF. There were 10 false positive analytes that resulted from unoptimized collision energy settings via RF-QTOF. There were no false negative samples via the RF-QTOF.

Conclusion/Discussion: This project was conducted to explore the possibility of using high-speed injection sequencing via the RF-QTOF as a screening platform for postmortem blood samples. True positives were able to be easily distinguished from false positives through comparison of high-resolution accurate mass spectral fragmentation data to that of known spectral patterns via a PCDL.

P07: Quantitation of 12 Cannabinoids and Metabolites in Postmortem Blood and Tissues by Liquid Chromatography Tandem Mass Spectrometry

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Background/Introduction: *Cannabis sativa*, commonly known as marijuana, is the most widely used illicit drug in the world with over 147 million people using the substance annually. Cannabis contains the psychoactive component, Δ^9-tetrahydrocannabinol (THC) and other phytocannabinoids including cannabidiol (CBD), cannabigerol (CBG), cannabinol (CBN), and Δ^9-tetrahydrocannabivarin (THCV). The metabolism of THC occurs through oxidation and hydroxylation to produce the metabolites 11-hydroxy-tetrahydrocannabinol (11-OH-THC), 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol acid (THC-COOH), 8β-OH-THC and 8β,11-di-OH-THC. THCV is metabolized to 11-nor-9-carboxytetrahydrocannabivarin (THCV-COOH). Conjugation is a common Phase II reaction that produces THC glucuronide and THC-COOH glucuronide.

Objectives: To develop and validate a quantitative liquid chromatography tandem mass spectrometry (LC-MS/MS) method for: THC, 11-OH-THC, THC-COOH, CBD, CBG, CBN, THCV, THCV-COOH, THC-COOH glucuronide, THC glucuronide, 8β,11-di-OH-THC, and 8β-OH-THC in postmortem blood and tissues.

Methods: To a 0.5mL blood sample or 0.5g tissue homogenate (1:4) sample, 25μL of deuterated internal standards was added followed by 3mL of cold acetonitrile. The sample was rotated for 15 min, then centrifuged at 3000rpm for 10 min. The organic layer was transferred to a clean centrifuge tube and then evaporated to approximately 750μL. The sample was pretreated with 2.25mL NH_4OH and 100μL of acetic acid prior to solid phase extraction with Agilent Bond Elut Plexa PCX 30mg columns. The columns were conditioned with 0.5mL methanol and then the samples were loaded. Columns were washed with 2mL of 79:20:1 water:acetonitrile:acetic acid and 200μL of hexane. The sample was eluted with 1.5mL of acetonitrile with 1% acetic acid, evaporated to dryness under nitrogen at 40°C, and then reconstituted with 100μL of initial mobile phase. Samples were analyzed using a Shimadzu LCMS 8040 (Columbia, MD). Chromatographic separation was performed using a Restek Raptor Biphenyl 2.7μm, 50x3.0mm column with a flow of 0.5mL/min. Mobile phase A was 0.1% formic acid in water and mobile phase B was 0.1% formic acid in acetonitrile and chromatographic separation was achieved with a gradient from 40% B to 65% B over 10 minutes. The extracted samples were analyzed in positive electrospray ionization mode with multiple reaction monitoring.

Results: The weighted $(1/x^2)$ calibration model for each compound was linear over the range of 0.25 – 50 ng/mL for THC glucuronide, 0.5 – 100 ng/mL for THC, 11-OH-THC, THC-COOH, CBD, CBG, and CBN, and 1 – 100 ng/mL for 8β-OH-THC, 8β,11-di-OH-THC, THCV, THCV-COOH, and THC-COOH glucuronide ($R^2 > 0.99$). Limit of detection and limit of quantitation were established as the lowest non-zero calibrator for each analyte. Percent accuracy at three concentrations ranged from 82% to 114%. The highest between-run precision (%CV) was 12.2% and all within-run precision values (%CV) were less than the acceptable criteria of 20%. No significant carryover or interferences with matrix, standards or commonly abused drugs were noted. Extracted samples were stable on the cooled autosampler (4°C) for at least 48 hrs. All analytes, except the glucuronides, met acceptable precision and accuracy criteria with extraction of a 1:2 and 1:5 diluted sample. Ion suppression/enhancement was evaluated in various matrices including blood, urine, vitreous humor, liver, lung, kidney, spleen, muscle, brain, and heart. Although many of the analytes exhibited suppression or enhancement in tissue samples, the factors did not affect the accuracy or precision of the method.

Conclusion/Discussion: The developed method quantifies cannabinoids, metabolites, and phase II glucuronide metabolites in human blood and tissues and will enable the evaluation of cannabinoid concentrations in postmortem biological specimens collected from pilots involved in aviation accidents.

POSTER

P08: Endogenous Gamma-Hydroxybutyrate Concentrations in Postmortem Samples

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Background/Introduction: Gamma–Hydroxybutyrate (GHB) is a naturally occurring short-chain fatty acid found in the human body as a catabolite of the neurotransmitter gamma-aminobutyrate. In the U.S., GHB has a history of being manufactured illicitly and abused but its prevalence has declined in recent years since DEA scheduling of the molecule. However, GHB has been a popular drug with misguided proposed benefits for the body-building and athletic community and a persistent party drug with reported GHB overdoses occurring worldwide. The interpretation of GHB in postmortem biological fluids carries complication due to the endogenous nature of the compound that often requires confirmation analysis to be performed on one or more biological matrices to detect exogenous exposure, typically in urine. The analysis is further complicated by the endogenous *de novo* production of GHB in postmortem specimens.

Objectives: This work sought to examine the prevalence of endogenous GHB concentrations in postmortem toxicology samples and identify suitable secondary matrices to determine exogenous GHB.

Methods: Samples were analyzed based on laboratory validated method using liquid-liquid extraction followed by gas chromatography-mass spectrometry with selective ion monitoring and an administrative LOD and LOQ of 10 mg/L. A sample size of 100 μL was used for blood, urine or vitreous humor and 200 μg was used for brain homogenate and liver homogenate samples. Blanks were analyzed using deionized water. 1.0 mL of internal standard containing 1 mg/mL GHB·Na d-6, 250 μL of cold 0.1 N Sulfuric Acid and 6.0 mL of Ethyl Acetate were added to all vials. Derivatizing agent of BSTFA (with 1% TMCS) was added after dry down of the organic layer for 15 minutes at 70 °C. Column used was a HP-1, 12 meters, with a constant flow mode and pressure of 6.6 psi at 65 °C. Oven temperature started at 65 °C for 0.5 min, ramped to 105 °C at 5°C/min, and then to 300 °C at 50 °C/min. The injector was set to a temperature of 200 °C in splitless injection mode. The mass spectrometer settings used a solvent delay of 4 minutes and an electron multiplier offset of 400 volts. The ions monitored at 50 milliseconds dwell time were: GHB: m/z 233, 204, 234 and GHB-d6: m/z 239 and the quantifying ratios were 233 and 239 m/z.

Results: Of the 348 cases analyzed, 39 cases resulted in positive GHB detection for heart blood with median concentration of 22.45 mg/L (range 10.4 - 62.16 mg/L). Table 1 shows the distribution of GHB heart blood positive cases and the subsequent identification of GHB in tissue samples; brain, liver, peripheral blood, urine and/or vitreous humor. Heart blood samples with no GHB detection were not selected to analyze for other tissues.

Table 1. Positive GHB samples, and associated detection in alternative matrices.

Tissues Type	Sample size	GHB Positive	Average Concentration (Range)	Median concentration
Heart blood	348	39	26.63 (10.40 – 62.16) mg/L	22.45 mg/L
Alternative matrices from GHB positive				
Peripheral Blood	21	18	19.18 (11.60 – 42.58) mg/L	15.29 mg/L
Liver homogenate	21	7	25.80 (10.82 – 40.63) mg/kg	23.48 mg/kg
Urine	16	1	10.31 mg/L	10.31 mg/L
Brain homogenate	21	Not detected	Not detected	Not detected
Vitreous Humor	21	Not detected	Not detected	Not detected

Conclusion/Discussion: A cut-off of 10 mg/L has shown to be inclusive of most endogenous GHB detection for this study. Urine has been studied extensively as a suitable matrix to confirm exogenous GHB due to its commonly low endogenous GHB concentration. However, urine samples are not always collected at autopsy. Our findings highlight the importance of multi-matrix analysis in postmortem toxicology for GHB interpretation. Our results suggest that vitreous humor and brain tissue are well suited to distinguish GHB intoxication from endogenous production.

P09: Method Validation for the Addition of Diphenhydramine, Doxylamine, Hydroxyzine, and Suvorexant to the Benzodiazepine and Z-Drug Quantitation Method for Blood, Urine and Tissues

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Background/Introduction: Common antihistamines like Diphenhydramine, Doxylamine, and Hydroxyzine may have side effects that can impair driving and contribute to an overdose. An in-house validated method currently quantitates for 19 Benzodiazepines and Z-drugs. Due to the frequency of qualitative detection for Diphenhydramine, Doxylamine, and Hydroxyzine, the lab decided to validate them for inclusion in the current quantitation method. Additionally the insomnia medications, Zaleplon, and Suvorexant were included to further expand the scope of the method. The validation of these 5 drugs will improve on laboratory throughput by analyzing over 20 drugs using one method in blood, urine, liver, brain, stomach contents, and vitreous humor.

Objectives: To validate the addition of Diphenhydramine, Doxylamine, Hydroxyzine, Suvorexant, and Zaleplon to a current LC-MS/MS Benzodiazepines and Z-drugs quantitation method.

Methods: A sample size of 250 μL or 0.25 g was used for standards, blank, and samples. Add 50 μL of internal standard containing 1000 ng/mL each of Estazolam-d5, Diphenhydramine-d3, Doxylamine-d5, Suvorexant-d6, and Zaleplon-d4. Add 750 μL of acetonitrile into each tube, vortex, and centrifuge for 5 minutes. Transfer supernatant into a clean test tube, then aspirate with DPX®WAX tips using a pneumatic extractor; holding liquid in tips for 15 seconds. Aspirate and dispense three times. Transfer 50 μL of aspirant into LC vial with 800 μL of initial mobile phase. Quantitative analysis was performed using a Waters Xevo TQ-S with an Acquity UPLC. The LC column was a Waters BEH C18 1.7 um, 2.1 x 100 mm, held at 40 °C, with an injection volume is of 7.5 μL. Aqueous mobile phase was 100 % water with 0.1% formic acid, and organic mobile phase was 100% acetonitrile with 0.1% formic acid. Starting conditions were 0.4 mL/min, 80% A, 20% B followed by a linear ramp at to 60% A, 40% B at 1.75 min, held to 3.50 minutes, a linear ramp to 15% A, 85% B at 5 minutes, linear ramp to 1 mL/min and 0% A, 100% B at 5.5 minutes, held to 7.5 minutes, and immediately changed to 0.4 mL/min and 80% A and 20 % B until 9.5 mintues.

Results: The method validation was done in accordance with SWGTOX, OSAC, and in-house lab validation guidelines for drug infusion, recovery, ion suppression/enhancement, method compound specificity, matrix interference, other drug interferences, calibration model, limit of quantitation, limit of detection, matrix limit of quantitation, bias, precision, instrument carry-over, dilution integrity, and extracted drug stability. Blank matrices used for the study were ante-mortem blood, post-mortem blood, liver homogenate, brain homogenate, stomach contents, urine, synthetic urine, and synthetic blood. Recovery studies were performed on all matrices and all recoveries were above 50% except for Suvorexant in central blood with 44%. A calibration model of quadratic $1/x^2$ with no forcing through zero was used for all 5 drugs. The calibration curve concentrations for Diphenhydramine, Doxylamine, and Hydroxyzine were 25 – 1600 ng/mL. Suvorexant and Zaleplon had a range of 4 – 256 ng/mL. Bias and precision produced acceptable results of less than 20% except in brain homogenate. Both bias and precision in brain exceeded 20% for all drugs except Suvorexant. Thus, brain homogenate was validated for qualitative identification only for Diphenhydramine, Doxylamine, Hydroxyzine, and Zaleplon. Carry-over was detected for Suvorexant at concentrations of above 1536 ng/mL but none was observed for other drugs.

Conclusion/Discussion: The addition of Diphenhydramine, Doxylamine, Hydroxyzine, Suvorexant and Zaleplon to a current Benzodiazepine and Z-drug method assisted in the rapid quantitation of previously only qualitative drugs for all matrices except for brain homogenate. The convenience of analyzing more than 20 drugs in one method allows laboratories to deliver quality results with a fast turnaround time.

POSTER

P10: Variation in Postmortem Production of Alcohol in Blood, Depending on Storage and Temperature Conditions

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Introduction: The stability of alcohol in blood sample drawn from a dead body depends on storage condition of the sample among many other factors. Storage condition includes addition of preservatives, temperature of the storage facility, headspace area within the storage container and proper sealing of the container. In this study we have studied two storage condition of the blood sample drawn from the dead bodies.

Objectives: Analysis of blood for ethanol quantification is the most frequently assay carried out in forensic toxicology. However, in cases of post-mortem collected samples, factors like preservation and tempreture play an major role. As Delhi, India faces a unique climate throughout the year, the ambient tempretures fluctuate between 4°C to 40°C. The following study compares the effect of preservation conditions and their effect on volatile ptofile in stored samples.

Methods: Permission to collect human samples was obtained from Institutional Ethical committee. 7ml of femoral blood was collected in 4 polypropylene sealed plastic bottlesfrom 180 cases. Every samples were divided into 4 sets and stored with different storage conditions of preservative use (with and without 2% sodium fluoride) and temperature of the storage (4°C and room temperature). 1ml from each of the sets was analysed on 0 day; 7th day; 14th day; 21st day; 28th day; 60th day; and 90th day. Analysis done just after collection without any experimental condition i.e. 0 day was treated as control. All the instrumental analysis was done using Gas-Chromatography Headspace. Trends of changes in ethanol concentration across the four conditions and for the different duration, as stated, was studied.

Results: Analytical results showed thatalcohol concentration at 0day was in the range of 21.37mg/dl- 501mg/dl (control sample). In the set1 (no preservation and stored at room temperature)ethanol level increased till 14th day after which the concentration declines even below the parent concentrationonfurther analysis. For set2 (with added preservative and stored at room temperature) there was an increase in ethanol concentration till 7thday after which it decreases, In set3 (with no preservative and stored at 4°C), there was an increases in concentrationtill 7th day which remains almost constant till 14th day after which it regressed. Samples of set4 (with added preservative and stored at 4°C) showed no change in the alcohol concentration throughout the duration of the study period.

Conclusion: The study provides the evidence that storage conditions affects the alcohol concentration of the blood sample. Addition of preservative and storage at 4°C is the best way to preserve the blood sample if cannot be analysed without delay. The second best condition is to preserve the sample at 4°C, even without the added preservative.

POSTER

P11: Prevalence of Designer Benzodiazepines: The New Lams and Pams

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Background/Introduction: Benzodiazepines are a class of drugs that are generally used for anxiety, panic attacks, and seizure prevention. In 2013, there are 13.5 million benzodiazepine prescriptions written. Due to their sedating effects, benzodiazepines are very popular among recreational users and contribute to a rising number of DUIDs in Alabama. Due to the propensity for recreational use, an increased prevalence of designer benzodiazepines has been detected in casework. Many designer drugs are formulations that were once developed within the pharmaceutical industry and have not been approved for use. Some are only legal in countries outside of the United States. Others have been designed and sold on the internet for recreational use.

Objectives: To validate a liquid-liquid extraction to identify 26 prescription or designer benzodiazepines by LC/MS/MS and monitor their prevalence in casework.

Methods: A liquid-liquid extraction was validated to expand our benzodiazepine scope and replace outdated, inefficient methodology. In addition to increasing the amount of prescription benzodiazepines, we also added several designer benzodiazepines to our panel. Deuterated internal standard, 6.0 mL n-butyl chloride, and 1.0 mL sodium carbonate were added to each sample. The samples were then rotated for 30 minutes and centrifuged for 15 minutes. The supernatant was dried down and reconstituted with methanol. The prepared samples were evaluated using an Agilent 6460 LC/MS/MS with a ThermoScientific Accucore biphenyl column. SWGTOX guidelines for accuracy, precision/bias, calibration model, ion suppression, dilution, interference, and stability were followed during the validation. We determined the prevalence of designer benzodiazepines in whole blood for DUI and postmortem casework. From late February 2019 to April 2019, we monitored designer benzodiazepines commonly detected in casework, alone and in combination with prescription benzodiazepines.

Results: The new method has the ability to quantitate 19 drugs and qualitatively identify an additional seven. The linear range was 10 ng/mL to 750 ng/mL with a linear or quadratic 1/x weighted calibration model based on regression analysis. The precision/bias and accuracy of all the targets had a %CV less than 20%. Lorazepam had the highest LOD at 5.0 ng/mL. The other drugs had LODs of 1.0-2.5 ng/mL. No interference was observed with different matrices or commonly used drugs. Ion suppression and enhancement were evaluated and had no significant effect (<20%).   The samples exhibited stability for up to 48 hours at 10° Celsius. All drugs had a maximum dilution factor of 4X with the exception of midazolam (maximum 2X dilution factor).

Sixteen instances (n=312) of designer benzodiazepines were identified and 75% of these cases also contained alprazolam (n=12). DUIs accounted for 75% of the cases, while the remaining four were post mortem cases. The most prevalent designer drugs detected were clonazolam (n=11), etizolam (n=3), and flubromazolam (n=2). In the cases with confirmed clonazolam approximately 88% had low concentrations of alprazolam (less than 20 ng/mL). There were three cases with etizolam, and two cases with flubromazolam. In 66% of the etizolam cases alprazolam was present in concentrations greater than 120 ng/mL. In one of the two flubromazolam cases, alprazolam was confirmed as well.

Conclusion/Discussion: The results indicate that clonazolam is being used in conjunction with alprazolam to get an enhanced pharmacological effect. There is also an indication of alprazolam use in conjunction with the etizolam and flubromazolam cases, however due to sample size this link cannot be definitively made. The prevalence of these designer benzodiazepines is closely associated with the ease of access via the internet. Forensic Toxicology laboratories should implement testing methods to detect designer benzodiazepines in routine casework due their increased abuse.

POSTER

P12: Merged Product Scan Screening by LC-MS/MS – A New Level of Spectral Confidence

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Background/Introduction: The Miami-Dade County Medical Examiner (MDME) Toxicology Laboratory processes more than 2,700 cases per year on a broad variety of post-mortem specimens. Over the years, an increasing number of novel psychoactive compounds have passed through preliminary screening undetected due to limitations in specificity and concentration of the initial immunoassay screen that is used in conjunction with GC-MS. To make up for these shortfalls, the laboratory must employ additional tests such as LC-Ion Trap-MS^n to complete the initial screen. This increases both turn-around-time and testing costs, and has prompted the laboratory to re-evaluate initial screening procedures. An alternative method would have to be more specific for identification, more sensitive to concentrations relevant to post-mortem analysis, and include a broader scope of targeted analytes.

Objectives: To explore the viability of replacing typical immunoassay testing with a more modern and specific assay. To seek out a technique for simple, yet confident post-mortem screening by LC-MS/MS that will be used in conjunction with a GC-MS screening technique without negatively affecting turn-around-time.

Methods: A Shimadzu LCMS-8060 triple quadrupole mass spectrometer was utilized in survey event mode. This data acquisition method monitors a single MRM for each analyte at a specified retention time. If the ion signal generated from the MRM exceeds a threshold, multiple product ion scans of the precursor ion are triggered. Each product ion scan is conducted at a different collision energy, resulting in different spectral patterns from the same precursor ion. The product ion scans are then merged together into a single spectra which is compared to an in-house library for identification purposes. The survey event acquisition mode is a function of the mass spectrometer, therefore independent of any sample preparation or chromatographic method used.

Results: The multiple product scan acquisition method is effective and can differentiate chemically similar drugs by their merged product scan spectra. Slight changes in fragmentation pattern can be detected and investigated due to the large amount of data acquired. More than ten scans per peak are collected even when drugs co-elute due to the 30,000 amu/s scan speed of the instrument. The in-house library created contains the most relevant analytes of interest in the laboratory including NPS, pharmaceuticals, and other toxins. The results were reproducible and reliable for identification. Sensitivity for most drugs investigated was below 2 ng/mL in blood. Each MRM event and its subsequent product ion scans can be finely tuned to meet concentration requirements or matrix effects by adjusting the trigger threshold, collision energy, dwell time, and scan speed.

Conclusion/Discussion: Utilizing LC-MS/MS for screening purposes can be even more beneficial when full spectral detail is generated. Relying on a typical three ion MRM spectra for identification can be misleading and may result in misidentification. While sufficient for quantitative analysis of previously identified substances, MRM's are not always reliable for screening. This is illustrated by the analysis of fentanyl analogues where the MRM spectral pattern is often virtually the same. These drugs are easily differentiated using merged product scanning. Merged product scans with library matching has created a new standard of identification for the MDME Toxicology Laboratory. When used in conjunction with a comprehensive GC-MS analysis, it provides a powerful initial screening protocol for post-mortem toxicology.

POSTER

P13: Comparison of Physicochemical Properties of Suvorexant with Quantitative Results using Authentic Samples

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Background/Introduction: Suvorexant (Belsomra®) is a novel dual orexin receptor antagonist (DORA) that is prescribed for the treatment of insomnia. Due to its long half-life (~12 hours) and the prominence of sedative hypnotic medications in driving impairment, drug-facilitated sexual assault, and death investigations, suvorexant is a drug of forensic interest. However, the prevalence of suvorexant in forensic samples is relatively unknown, and many physicochemical properties of the drug have not been studied. In fatalities, interpretation of results is complicated by postmortem redistribution, which is heavily influenced by the physicochemical properties of the drug. Currently, limited information is available concerning the distribution of suvorexant in biological specimens of forensic interest. The goal of this study was to further characterize the physicochemical properties of suvorexant to help determine its potential for postmortem distribution, and to compare this information with data from authentic case samples.

Objectives: To experimentally determine blood/plasma (B/P) ratios and LogP values for suvorexant and compare results with suvorexant in authentic case samples.

Methods: Liquid chromatography-quadrupole/time of flight-mass spectrometry (LC-Q/TOF-MS) was used for the quantitative identification of suvorexant. Samples from adjudicated casework were obtained from participating laboratories. Suvorexant was isolated from 0.5 mL of specimen using a simple acidic/neutral liquid-liquid extraction. Estazolam-D5 was used as the internal standard in the absence of a commercially available isotopically-labeled suvorexant at the time of the study. An Agilent Technologies 6530 Accurate-Mass LC-Q/TOF-MS equipped with electrospray ionization (ESI) was used for quantitative analysis. Suvorexant was identified in antemortem blood, postmortem peripheral and heart blood, and vitreous humor using a previously validated method. Suvorexant metabolites were identified in authentic samples using high resolution mass spectra, MS/MS fragmentation patterns, and in-vitro generated metabolite controls using recombinant CYP450 isozymes. The B/P ratio for suvorexant was determined experimentally, and partition coefficients (Log P values) were evaluated using various buffer systems over a range of pH values and ionic strengths.

Results: Suvorexant was identified in both antemortem and postmortem blood specimens. For specimens containing low concentrations of suvorexant, the M9 (hydroxylated) metabolite was identified. In the absence of commercially available standards, mass accuracy, MS^2 spectra, and retention time matching using in-vitro generated metabolites were utilized. Concentrations of suvorexant in vitreous fluid were negligible, but the partitioning characteristics of suvorexant studied herein help to explain this. There were no significant differences in suvorexant Log P values over a range of pH and ionic strengths. Experimentally determined partition coefficients were in good agreement with theoretical values generated using software. The drug consistently partitioned into organic solvent, further demonstrating the lipophilicity of the drug.

Conclusion/Discussion: Interpretation of postmortem toxicology results is highly dependent on physicochemical properties of the drug as well as its distribution within the body after death. In order to better predict how a drug will behave after death, an understanding of its lipophilicity, volume of distribution, and metabolism is essential. In addition, drug concentrations may be influenced by the sampling site which demonstrates the need to characterize compounds in a variety of authentic specimens. This study aims to help expand knowledge of suvorexant distribution in various specimens of forensic interest.

P14: Comparison of Blood and Vitreous Fluid Drug Analytes in Opioid Overdoses

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Introduction: Vitreous fluid has been routinely used to test for ethanol in postmortem cases, especially where there is a question about the integrity of blood samples due to decomposition or contamination. Additionally, vitreous fluid has been used to evaluate electrolytes and glucose to provide information for the interpretation of cause of death. Due to the limited amount of sample, there has not been a lot of interest in analyzing vitreous fluid for drugs. Utilizing the analytical capabilities of current technology, we have developed and validated an assay for fentanyl and the metabolites of heroin. Heroin and fentanyl deaths have increased significantly over the last several years. Postmortem analysis of blood and urine are typically utilized to interpret the cause of death in heroin overdoses. Frequently due to the circumstances of the overdose and the time of death blood and urine samples only reveal the presence of morphine. Analysis of vitreous fluid in suspected opioid overdoses has shown that 6-acetylmorphine (6-AM) can be detected in vitreous fluid when not found in the postmortem blood of a heroin overdose. Tracking and evaluating vitreous fluid and blood concentrations of morphine and 6-AM may provide a way to determine the time of drug exposure and time of death.

Objective: To develop and validate a vitreous fluid assay by LC-MS/MS and compare heroin metabolites in blood samples collected in postmortem cases involving fatal opioid overdoses.

Methods: A selected reaction monitoring (SRM) method on a Thermo-Fisher Vanquish UHPLC coupled to an Endura Triple-Quadrupole Mass Spectrometer (LC-MS/MS) was used to analyze and identify 6-AM and morphine in vitreous fluid and postmortem blood. Validations of blood and vitreous fluid opioid assays provided LOQs of 0.5 ng/mL for both 6-AM and morphine. Sample preparations for vitreous and blood were carried out using a solid phase phospholipid removal step followed by dry down and reconstitution into 95:5 ratio of H_2O:MeOH. 3 μL of reconstituted extract was run on a Phenomenex kinetex C18 column (2.6 μm, 2.1 x 100 mm). Gradient elution consisted of 10 mM Ammonium Formate Aqueous buffer (pH 4.0) and Acetonitrile ramped from 0% organic to 50% over 5 min. Analytes detected by a selected reaction monitoring (SRM) method and quantified between 0.5 and 500 ng/mL.

Results: Twelve postmortem cases were analyzed for both 6-AM and morphine in whole blood and vitreous matrices (**Table 1.**) The percentage of cases where morphine was quantified above 0.5 ng/mL but 6-AM was undetected in blood matrices was 33.3%. However, if vitreous analysis for 6-AM was included, then the percentage of cases where heroin use was unambiguously identified raised to 83.3% suggesting that including vitreous matrix in routine opioid analysis more than doubled the correct attribution for cause of death. Moreover, in 40% of those 6-AM positive cases, identification of 6-AM required a sensitivity of < 1.0 ng/mL. When morphine was observed in blood, it was also observed in vitreous matrix 100% of the time.

POSTER

Table 1. Quantitative Results (ng/mL)

Case #	Vitreous 6-AM	Blood 6-AM	Vitreous Morphine	Blood Morphine
1	21.4	8.9	12.8	123
2	2.1	ND	5.1	3
3	0.7	ND	0.7	6.1
4	0.6	ND	3.6	3.2
5	0.5	ND	2.9	2.8
6	21.5	20.2	17.5	139.6
7	ND	ND	105.8	511.8
8	15.4	5.1	24.8	49.7
9	ND	ND	9.1	32.8
10	14	5.8	17.4	89.2
11	ND	ND	2.4	6.5
12	1	ND	10.4	16.1

Conclusion/Discussion: Using a sensitive LC/MS/MS enables the detection of drug metabolites in vitreous fluids that may not be present in blood in a postmortem case. Identification of trace 6-AM in vitreous fluid analysis allows for definitive identification of heroin use with a questionable cause of death in opioid cases.

P15: The Effect of Washout on Drug Concentration in Interred Remains

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Background/Introduction: In forensic toxicology casework, the primary matrices include biological fluids, such as blood and urine. However, some forensic casework includes the testing of decomposed postmortem tissue samples. Forensic toxicologists therefore need to know if decomposition has an effect on the identification and quantification of a drug because any analysis completed by the toxicologist assists the forensic pathologist in determining the drug(s) impact on the cause of death. This involves the understanding of taphonomy and its effects. In buried remains this process becomes even more complicated due to the microenvironment of the burial site.

Objectives: This research aims to determine the relationship between ante mortem amphetamine dose, the concentration of amphetamine and its metabolite norephedrine in tissue and the stage of decomposition of buried remains. It also aims to determine the impact of burial location.

Methods: In order to determine these relationships, seventeen male Long Evans rats were dosed with amphetamine at varying concentrations once a day for ten days. For each dose at 10 mg/kg, 6 mg/kg, and 2 mg/kg four rats were used per injection type. The 1 mg/kg and 0.2 mg/kg dose used 2 rats per dose type. The 0.6 mg/kg dose was only performed on 1 rat. The rats dosed at the 10 mg/kg, 6 mg/kg, 2 mg/kg concentrations were euthanized with CO_2 ten days after the final injection while the other doses were euthanized with CO_2 immediately following the final dose. The rats were then interred in the New Jersey Pine Barrens and then later exhumed at different stages of decomposition in accordance with the Megyesi method. Following exhumation, liver, heart and brain tissue samples were collected during dissection, but where decomposition was more advanced, samples were collected from the general location of the organs. The samples were stored at -20°C until analysis. During analysis the samples were tested for amphetamine and its metabolite norephedrine.

The samples were prepared by homogenizing the tissues with saline using a Biotage® Bead Ruptor 24 (1mL:1g for brain and liver, 2 mL:1 g for heart) and performing a liquid-liquid extraction for amphetamine and norephedrine. The analytes were identified and quantified using a Perkin Elmer Clarus® SQ 8T gas chromatograph-mass spectrometer (GC-MS) in SIM (selected ion monitoring) mode with amphetamine-D11 and norephedrine-D3 as the internal standards.

Results: The analysis of the postmortem tissue samples showed that amphetamine and norephedrine can be detected in soft tissue through the different stages of decomposition. It was also shown that there is no correlation seen between tissue concentration and initial dose, there is no correlation between tissue concentration and interment time, and there is no correlation between delayed and immediate euthanisation. This was shown through the comparison of the results of each rat. For example, the concentration of a rat dosed at 6 mg/kg had a higher concentration than that of a rat with an earlier exhumation and that of the higher dosed rats. The positive unburied controls had substantially higher tissue concentrations indicating that burial has an effect on concentration. It was also hypothesized that the microenvironments of each burial site may have affected the levels of amphetamine and norephedrine in the tissue, and that washout from rain water may have occurred. The results show that negative samples do not necessarily indicate that no drug was ingested prior to death.

Conclusion/Discussion: Decomposition and interment have an overall effect on the detection and quantification of amphetamine and norephedrine from tissues. We found that negative toxicology results do not always indicate that no drug was ingested. Therefore, forensic toxicologists need to take in consideration the length of decomposition, the environment in which the decomposition occurs, and the tissue type when analyzing for amphetamine in postmortem interment cases.

Keywords: Amphetamine, post-mortem toxicology, interment

POSTER

P16: Detection of Common Adulterants in Opioid Involved Overdose Deaths, CDC's State Unintentional Drug Overdose Reporting System, July 2016-June 2018

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Background/Introduction: The opioid overdose epidemic has resulted in nearly 400,000 deaths from 1999-2017. The changing nature of the opioid overdose epidemic has called for a comprehensive and enhanced approach in targeting prevention and response efforts. Efforts such as enhanced surveillance of opioid overdose deaths are instrumental in characterizing the nature of these deaths. Most notably, access to toxicological findings for these decedents can be useful in not only understanding the specific substances contributing to the death, but also identifying common adulterants or 'cutting agents' that may potentiate toxic side effects. Adulterants, typically used to bulk up the drug and increase dealer profits, can sometimes mimic its effects or mask the impurity of the drug.

Among illicit opioids (e.g. heroin), the most commonly observed adulterants have been caffeine and quinine. Substances reported in drug seizures tested by forensic laboratories are typically restricted to controlled substances, resulting in underreporting of non-controlled adulterants. Using postmortem toxicology findings to detect common adulterants may not only help address underreporting but also aid in characterizing the local illicit drug markets.

Objectives: Describe the prevalence of two common opioid adulterants, caffeine and quinine, in postmortem toxicology findings for opioid-involved overdoses.

Methods: CDC's Enhanced State Opioid Overdose Surveillance (ESOOS) program funds 32 states and the District of Columbia to capture data on non-fatal and fatal opioid overdoses. Data from death certificates, medical examiner and coroner (ME/C) reports, and toxicology findings are abstracted into the State Unintentional Drug Overdose Reporting System (SUDORS). Elements abstracted include postmortem toxicological testing results to capture substances detected as well as those determined by an ME/C to contribute to death. The distribution of two common adulterants associated with illicit opioids, caffeine and quinine, an antimalarial, was examined among decedents with complete toxicology reports.

Results: During the study period, quinine and caffeine were detected among all opioid-involved overdoses in approximately 2% and 11% of cases, respectively. When examined by month, these percentages ranged from a low of 1% to a high of 3%, for quinine and from 5% to 16% for caffeine. In addition, there was considerable variability across jurisdictions. Among all opioid-involved overdose deaths, quinine was detected most frequently in North Carolina (13%), and caffeine highest in Maine (71%). When examining deaths where heroin was listed as the cause of death, quinine was noted in 3% of cases compared to 11% of cases for caffeine. Results were similar among fentanyl-involved overdose deaths. Among states, Illinois and North Carolina had the highest proportion of heroin-involved overdose deaths where quinine was also present, both at 17%, whereas caffeine detection was highest in Vermont (74%) and Maine (79%). When examining fentanyl-involved overdose deaths across jurisdictions, the highest detection of quinine was in North Carolina (21%) and the highest detection of caffeine in Maine (75%).

Conclusion/Discussion: Findings from these analyses revealed that two common adulterants for opioids, caffeine and quinine, were seen in less than 12% of all opioid-involved overdose deaths. However, there was notable geographic variation in their distribution, with the highest proportion occurring in the North Carolina and Maine. Variability in the detection of adulterants could indicate differences in local drug markets, potentially aiding in the identification of trafficking routes. Additionally, information on the local prevalence of cutting agents could aid in the management of acute intoxications, as these adulterants may have harmful health effects. Although numerous forensic laboratories perform testing on drugs seized, the presence of the adulterants is not routinely reported. Utilizing additional data, such as toxicology findings, can help fill in critical gaps in characterizing local drug supply, as well as better inform both law enforcement and health care providers.

POSTER

P17: Retrospective Review of Phencyclidine in Cases Submitted to the New York City Office of Chief Medical Examiner Between 2003 and 2018

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Background/Introduction: Phencyclidine (PCP) is a dissociative drug that produces an anesthetic effect and was originally developed for that purpose but discontinued for use in humans. Although an increase in the use of PCP in the United States was reported from 2005 to 2011, overall use has remained low nationwide. In the 2017 National Household Survey on Drug Use and Health, 6 million U.S. residents aged 12 and older reported using PCP at least once in their lifetime, down from 6.4 million in 2016.

There are significant regional differences relating to the use of PCP nationwide but also within the State of New York. This along with the increase in novel psychoactive substances (NPS), and the ongoing opioid epidemic, have changed the landscape of commonly misused drugs. A retrospective review of PCP positive cases submitted to the New York City Office of Chief Medical Examiner (OCME) between 2003 and 2018 was undertaken to better understand changing trends in the use of PCP in New York City (NYC).

Objectives: A greater understanding of the changing use of PCP in NYC through a review of post-mortem, driving under the influence of drugs (DUID) and drug facilitated sexual assault (DFSA) cases.

Methods: The NYC OCME provides forensic toxicology services for all five boroughs of the City of New York (population 8.6 million). In-house case management and laboratory information management systems were utilized to collate all cases where PCP was detected over a period of 16 years from 2003 to 2018 for postmortem cases and a period of 10 years from 2009 to 2018 for DUID and DFSA cases. Averaging over 5,500 cases per year, many case files were manually reviewed to identify PCP positive cases where a database did not exist. Demographic information recorded included sex, race, age, gender, cause and manner of death and other drugs detected.

Analysis of PCP over the study period involved screening by gas chromatography-mass spectrometry (GCMS) with quantification by GC-NPD or GCMS SIM. The lower limit of quantification was 0.025mg/L.

Results: Between January 1st 2003 and December 31st 2018, PCP was identified in a total of 496 deaths reported to the NYC OCME. 85% were male, with Black, Hispanic and White individuals accounting for 41.7%, 35.7% and 21.3% respectively. Manner of death was ruled an accident in the majority of cases (58.8%, N=292) with intoxication the leading cause of death overall (49.1%, N=244), followed by homicide at 20.1% (N=100) and gunshot wounds the second leading cause of death (13.7%, N=68). Over the 16-year period, the number of postmortem cases with PCP varied from as few as 9 in 2013 to 47 in 2011 (mean = 31; median = 32).

Over the 10-year period from 2009 to 2018, a total of 102 DUID and 25 DFSA cases contained PCP. Of the DUID cases 95% involved males while 100% of the DFSA cases were female. Unlike the deaths, PCP-positive drivers were most commonly Hispanic (43.7%), White (33.3%) and Black (22.9%).

Table 1 summarizes the PCP concentration ranges measured across the categories of manner of death, DUID and DFSA.

Table 1: Phencyclidine blood concentration ranges (mg/L)

Accident	Homicide	Suicide	Natural	Undetermined	DUID	DFSA
<0.025 – 1.3	<0.025 – 0.37	<0.025 – 1.6	<0.025 – 0.33	<0.025 – 0.97	<0.025 – 0.11	<0.025 – 0.09

The most common drugs found in combination with PCP, in order, were cocaine, opiates, cannabis and ethanol.

Conclusion/Discussion: The number of cases identified each year that contained PCP fluctuated over the study periods for all case types but appears unaffected by the recent increase in alternative NPS. The manner of death most commonly reported with PCP detected was accident (58.8%) and homicide (20.1%).

P18: Quantitative Analysis of 58 Antipsychotics and Antidepressants in Human Urine by LC-MS/MS

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Background/Introduction: Human mental health disorders have become modern-day living diseases and contribute significantly to worldwide morbidity and mortality. This is reflected in fast growing numbers of antipsychotics (APs) and antidepressants (ADs) entering the market and the rising rate of prescription. In the forensic setting, due to their abusive potential, the detection of these drugs is critical in determining their involvement in intoxications and suicides. In the clinical setting, analysis of APs and ADs in blood or urine is necessary to ensure suitable therapeutic concentration and to monitor patients' compliance. It has been reported that the patient's adherence to prescribed APs is quite low, and thus new clinical recommendations have been released regarding when and how to use urine testing procedures to help monitor adherence. By combining a simple sample preparation procedure and a fast chromatographic elution with the Raptor Biphenyl column, a highly specific and accurate method was established for simultaneous measurement of 58 AP and AD drugs in human urine.

Objectives: Develop a comprehensive method for simultaneous quantification of 58 AP/AD drugs and metabolites using the combination of a simple urine hydrolysis procedure and a fast chromatographic method.

Methods: Drug-free human urine (BioIVT) was fortified with 58 analytes to prepare the calibration standards and QC samples. Bupropion-D9 was used as the internal standard for quantification of all 58 compounds. Because these drugs types undergo phase II metabolism, sometimes complex hydrolysis procedures are implemented to convert all to their parent forms. The urine sample was treated with IMCSzyme® for hydrolysis (45°C for 30 minutes) followed by protein precipitation with acetonitrile. The supernatant was injected onto a Raptor Biphenyl 2.7 μm, 50 mm x 3.0 mm column for analysis using a Shimadzu Nexera X2 LC System coupled with a SCIEX Triple Quad 4500 MS/MS.

Results: In addition, the existence of isobaric compounds (maprotiline vs. amitriptyline; protriptyline vs. nortriptyline) is an added difficulty as chromatographic separation is required for these analytes. By specifically evaluating these issues, a gradient elution was designed to greatly reduce the carryover while maintaining chromatographic separation of the isobaric compounds. Using additives of 0.1% formic acid and 5mM ammonium formate in both aqueous and organic (methanol) mobile phases, 28 APs and 30 ADs could be analyzed with a total run time of 5.5 minutes with no matrix interference.

The linearity test showed that the majority of compounds (43 out of 58) could be quantified in the range of 10-2500 ng/mL with either quadratic or linear regression (1/x weighted). Other compounds could be quantified in the range of 20-2500 ng/mL, 10-500 ng/mL, or 10-1000 ng/mL. Chromatographic carryover was initially problematic for accurate measurement of AP and AD drugs. Due to their relatively high carryover, olanzapine and desmethylolanzapine were quantified in the range of 25-2500 and 35-2500 ng/mL, respectively. All compounds showed good linearity with r values of 0.998 or greater, and the % deviations were <15%. Three QC levels (25, 200, 1000 ng/mL) were prepared at suitable concentration per the different quantification ranges of the compounds. The method accuracy was demonstrated with %recovery <15% of the nominal concentration for all QC levels. The %RSD was <10% for acceptable method precision.

Conclusion/Discussion: It was demonstrated that simultaneous measurement of 58 AP and AD drugs and their metabolites in urine can be achieved with a simple sample preparation procedure and a fast 5.5-minute LC-MS/MS analysis using the Raptor Biphenyl column. The major carryover issue was addressed and resolved in this study with proper injection needle rinsing and LC elution conditions. The established method provides high-throughput and accurate determination of the majority of mental health drugs on the market, and is suitable for both clinical and forensic monitoring of AP and AD drugs in human urine.

POSTER

P19: Analysis of Phosphatidylethanol in Human Whole Blood by LC-MS/MS

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Background/Introduction: Phosphatidylethanol (PEth) is a group of phospholipids formed through enzymatic reaction between ethanol and phosphatidylcholine on the cell membrane. Among multiple homologues of PEth, PEth-16:0/18:1 (palmitic acid/oleic acid) is the predominant molecule extracted from human erythrocytes and can be measured in whole blood as specific biomarker of alcohol consumption with a detection window of up to 3-4 weeks. Previously PEth was considered a biomarker for high and sustained alcohol consumption, but with the application of highly sensitive LC-MS/MS techniques, it is now possible to use PEth concentration in blood to differentiate chronic drinking from social drinking or as a marker of absolute abstinence. In this study, a fast chromatographic analysis was developed using a Raptor FluoroPhenyl column. Specific and sensitive measurement of PEth-16:0/18:1 in whole blood was achieved with a combination of simple protein precipitation and fast 3.5-minute LC cycle time.

Objectives: The intent of this study was to develop a fast chromatographic analysis of PEth-16:0/18:1 in whole blood using a Raptor FluoroPhenyl column. Specific and sensitive measurement of PEth-16:0/18:1 in whole blood was achieved with a combination of simple protein precipitation and fast 3.5-minute LC cycle time.

Methods: The PEth-free pooled human whole blood (BioreclamationIVT) was fortified with PEth-16:0/18:1 (RedHot Diagnostics AB) to prepare calibration standards and QC samples. The linearity ranges were from 0.025 - 4µM (18-2810 ng/mL). Three QC levels were prepared at 0.075, 0.75, and 2.5µM. Following the sample preparation procedure described by RedHot Diagnostics, the blood sample (50µL) was mixed with 50µL of internal standard (0.4µM PEth-d5 in 2-propanol) and 150 µL of 4:1 2-propanol: tetrahydrofuran. The mixture was vortexed for 20 seconds at 3000rpm and centrifuged for 10 minutes at 4300rpm. The supernatant (2µL) was injected onto a Raptor FluoroPhenyl 2.7µm, 50x2.1mm column and analyzed using a Waters ACQUITY UPLC coupled to a Xevo TQ-S MS/MS system. The separation was performed using 5mM ammonium acetate in water and 9:1 methanol: 2-propanol as mobile phases with a 3.5 minute cycle time.

Results: Detection range was established from 0.025 to 4µM with standard curves showing r2 values of 0.999 or greater and the %deviations (from nominal concentration) were <10% (<20% for 0.025 µM standard). The method accuracy was demonstrated from the %recovery of within 5% of the nominal concentration for all QC levels. The %RSD was from 0.117-1.33% and 2.30-5.08% for intra-day and inter-day, respectively, indicating acceptable method precision. Consistent chromatographic performance (retention, peak shape, and sensitivity) was observed upon continuous 500 injections demonstrating good method robustness.

Conclusion/Discussion: The analytical conditions outlined in our study indicate that the method was specific and sensitive for PEth analysis in human whole blood. The accurate and reproducible analysis can be achieved with a simple protein precipitation procedure and a fast 3.5 minutes of chromatographic run time. This method is thus applicable for low-cost and high throughput analysis to monitor alcohol consumption.

POSTER

P20: Analysis of d and l-Amphetamines Using Reversed-Phase LC-MS/MS

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Background/Introduction: Amphetamine (AMP) and methamphetamine (MAMP) are psychostimulant drugs and occur as two enantiomers, dextrorotary and levorotary due to their chiral center. The dextro-methamphetamine (d-isomer) form is highly abused and typically found in illicit preparations. However, detection of abuse is complicated because consumption of some over-the-counter and prescription medications may yield positive results if the analytical method cannot distinguish between the enantiomers. Chiral separation of d- and l-methamphetamine and their metabolites d- and l-amphetamine can help determine whether the source was licit or illicit, but chiral columns can be expensive, may necessitate a dedicated instrument, and are not as broadly useful as ubiquitous C18 columns.

Objectives: The intent of this study was to develop and validate a high throughput LC-MS/MS method for the separation and quantitation of l- and d- methamphetamine and amphetamine enantiomers in urine, using a reversed-phase column with pre-column derivatization followed by dilution in urine, with a total run time of 7 minutes.

Methods: In order to provide labs with a high-throughput assay that effectively separates d- and l- amphetamine and methamphetamine enantiomers in urine without the use of a costly and specialized chiral column, a LC-MS/MS method was developed using a standard reversed-phase Raptor C18 column. The method employs a simple pre-column derivatization followed by dilution and results in a selective, specific analysis of d- and l-amphetamine and methamphetamine enantiomers that is free from sample matrix interferences. The LC-MS/MS method was developed using a Raptor C18 2.7 µm, 100 mm × 2.1 mm column with water and methanol mobile phases modified with 0.1% formic acid. The LC gradient resulted in a total analysis time of 7.0 minutes, including column equilibration time with good baseline resolution of the target compounds, allowing easy peak identification and quantitation. Carryover was not observed.

Results: Separation was achieved within a total run time of 7 minutes and quantitation in urine was performed across a linear range of 50-5000 ng/mL. Validation across this range demonstrated good linearity with r^2 values of 0.998 or greater and acceptable method accuracy and precision with %RSD <10% and %recovery within 10% of the nominal concentrations for low, mid, and high QC levels and within 15% for the LLOQ. This method provides reliable analysis of d- and l-amphetamine and methamphetamine enantiomers in a workflow and time frame suitable for high-throughput clinical and forensic toxicology labs.

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Conclusion/Discussion: An enantioselective method for d- and l-methamphetamine and amphetamine determination in urine was successfully developed. To accomplish AMPs chiral resolution, pre-column derivatization with CDR (Marfey's reagent) was performed with minimal (dilute and shoot) sample preparation. Although CDR utilization in the present method increased the sample preparation time by approximately 2 h, conventional reversed-phase conditions on a C18 column were utilized and the chromatographic separation time was limited to 7 min. Additionally, the use of deuterated internal standards for each enantiomer allowed the method to maintain accurate and reliable quantitative results and able to accurately identify/ quantify licit vs. illicit methamphetamine.

P21: A Novel Solution for EtG/EtS Analysis in Human Urine by LC-MS/MS

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Introduction: Ethyl Glucuronide (EtG) and Ethyl Sulfate (EtS) are stable phase II metabolites of ethanol and unique biomarkers for alcohol intake and abuse. Analysis of these markers offers many advantages for abstinence monitoring including a longer detection window, stability in stored specimens, and specificity. EtG and EtS are polar analytes, making them difficult to retain via reversed-phase chromatography. Both compounds are also very sensitive to matrix interferences which can result in being unable to achieve low limits of detection. In some troublesome urine samples, isobaric interferences elute close to EtG and EtS, resulting in poor peak shapes and ultimately leading to potentially biased results. In this study, a simple solvent precipitation method was developed for the analysis of EtG and EtS in human urine by LC-MS/MS.

Objectives: The intent of this study was to develop and validate a method that provides a simple, fast, and sensitive measurement of EtG and EtS in human urine.

Methods: MS gold human urine (pre-screened to confirm absence of EtG/EtS) was fortified with EtG and EtS ranging from 50-5,000 ng/mL for both analytes. MS gold human urine calibrators and QC samples, blank pooled human urine spiked at QC concentrations and UTAK level 1 and 2 QC samples were prepared using a simple solvent precipitation method, where a 50 µL aliquot is taken from the spiked samples, mixed with internal standard and 150 µL of acetonitrile, vortexed at 3000 rpm for 10 seconds and centrifuged at 4300 rpm for 10 minutes at 10 °C. After centrifugation, 100 µL of the supernatant was diluted with 900 µL (40-fold dilution) of 0.01% formic acid in water and injected on a Raptor EtG/EtS column (100x2.1mm, 2.7 µm). The mobile phases used were 0.01% formic acid in water (aqueous phase) and 0.1% formic acid in acetonitrile (organic phase) and the chromatographic separation was achieved with a gradient elution of 5-35% organic phase in 3 minutes with a total analysis time of 4.5 minutes, including column re-equilibration time. Experiments were performed on both a Shimadzu Prominence HPLC coupled with a SCIEX API 4000™ and a Shimadzu Nexera X2 UHPLC coupled with a Shimadzu LCMS-8045 using electrospray ionization in negative ion mode.

Results: EtG and EtS were successfully resolved from matrix interferences in troublesome urine samples. The method sensitivity was significantly improved with sample cleanup, decreased formic acid concentrations in the aqueous mobile phase, MS source parameters optimization and source cleaning, thereby making the method accessible for older instruments with lower sensitivity. Excellent calibration linearity was achieved for both analytes with R^2 values ≥ 0.998 and % deviation < 12.0%. Four levels of QC samples were analyzed for accuracy and precision across multiple days, and column lots. Mean accuracy values ranged from 93%-105% of the nominal concentration, for the QC LLOQ, low, mid, and high samples and 98-133% for both UTAK level 1 and 2 QC samples for both analytes. The %RSD did not exceed 10% for any set of QC samples throughout the study.

Conclusion: A simple protein precipitation method was developed for the quantitative measurement of EtG and EtS in human urine. The analytical method was demonstrated to be fast, reproducible, and rugged.

POSTER

P22: Streamlining Unknown Screening for Postmortem Analysis

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Background/Introduction: Accurate identification of drugs present in postmortem samples is critical for forensic toxicologists to successfully carry a case examination. The use of high resolution mass spectrometry (MS) in the forensic laboratory enables toxicologists to rapidly obtain complete chemical profiles from biological samples, which subsequently leads to increased confidence in compound identification through accurate mass information at low analyte concentration.

Objectives: The objective of this study is to enable confident unknown substance identification within an efficient, all-in-one workflow. This comprehensive drug screening workflow should enable reliable compound fragmentation comparison to library spectra for confident drug identification and retrospective analysis to avoid missing potential drugs present in postmortem samples.

Methods: The 151 target analytes present in this panel include a variety of novel psychoactive substances (NPS) as well as other commonly prescribed drugs of abuse. Control whole blood samples were spiked with a 1 µg/mL stock standard solution mixture and extracted for LC/MS screening to determine the retention times. Forensic case postmortem blood samples were extracted by using a protein precipitation procedure followed by centrifugation; supernatant was evaporated and reconstituted in mobile phase for analysis.

Analytes were chromatographically separated at 30˚C using a Phenomenex Kinetex phenyl-hexyl (50 x 2.1 mm, 2.6 µm) column. Mobile phases were water and methanol with appropriate additives, 1 mL/min flow rate. Mass spectrometric detection was conducted on a X500R QTOF System operated in positive electrospray mode using SWATH˚ Acquisition SWATH Acquisition enabled to collect MS and MS/MS information on every detectable peak within a sample, essentially creating a digital record of each sample analyzed. Samples were evaluated against four main confidence criteria weighted as follows: mass error (15%), retention time (30%), isotope ratio difference (5%), and library score (50%) for all compounds. These criteria were used to generate a combined score. The processing criteria for positive identification of an analyte in a sample required all four main confidence criteria to pass.

Results: All 151 target analytes spiked control whole blood samples were successfully detected at 1 µg/mL . Their retention times and MS/MS quality spectra were used to build a data analysis processing method and high resolution spectral libraries for the postmortem case samples. SWATH˚ Acquisition generated comprehensive and high-quality MS/MS spectra, enabling reliable compound fragmentation for spectral library database searching for the analytes present, minimizing the risk of missing potential forensic compounds present in postmortem samples. Postmortem case samples were examined, and a number of targeted compounds were successfully identified and quantified above the LOD in the first round of data processing. Next, the same sample was re-interrogated for the presence of a potential known NPS (i.e., Fentanyl), by extracting the compound's molecular formula ($C_{21}H_{26}N_2O$). Based on the confidence criteria set in SCIEX OS Software Fentanyl was detected with good confidence in the interrogated sample. This highlights the ability for users to retrospectively analyze previously acquired SWATH data sets and screen for new compounds without having to re-inject samples, when newly identified forensic targets are discovered. Table 1 shows a summary of the target analytes detected in a postmortem case sample. A more thorough description of the compounds detected in postmortem case samples will be provided in the poster.

Conclusion/Discussion: The implementation of a robust method development process resulted in high combined scores for all compounds based on the four main confidence criteria defined in the processing method. These criteria were automatically calculated for all the forensic compounds detected in postmortem samples and visualized using a traffic light pattern on the software, such that compounds identified with high confidence are indicated using green check symbols. These traffic lights can be sorted and filtered by identification criteria for review and used to positively report identified compounds. Additional quantification was

implemented by setting an analyte concentration threshold based on the LODs to minimize false positives and/or false negative hits, which resulted sub ng/mL LOD for the majority of the drugs screened.

Overall, the developed QTOF-MS screening approach enabled the rapid implementation and optimization of the screening workflow for 151 compounds of interest for confident drug identification and retrospective analysis to avoid missing potential drugs present in postmortem samples.

Table 1. Summary Table for Postmortem Blood Sample for Case Sample #3. Inter-day average (n=3) for the detection of 10 compounds screened in a postmortem blood sample.

Drug Detected	Concentration (ng/mL)	Library Score (%)	Combined Score (%)
Acetamino-phen	773.162 ± 7.4	100	81.3 ± 0.2
Codeine	24.290 ± 2.1	95.7	89.5 ± 0.1
Diazepam	74.819 ± 2.4	98.3	94.6 ± 0.6
Diphenhydr-amine	56.226 ± 1.2	100	94.9 ± 1.1
Fentanyl	7.835 ± 0.3	100	90.3 ± 0.8
Hydrocodone	6.499 ± 0.4	95	79.3 ± 0.5
Hydromor-phone	4.716 ± 0.1	96.4	85.4 ± 1.2
Morphine	29.732 ± 1.8	89.8	73.2 ± 0.5
Naloxone	114.523 ± 3.4	100	82.8 ± 1.2
Nordiazepam	122.151 ± 1.4	100	90.1 ± 0.4

P23: Improving Analytical Throughput Using Automated Carryover Monitoring

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Background/Introduction: MPX[TM] technology from SCIEX allows multiplexing of two LC streams with a single mass spectrometer. This technology is frequently used in laboratories for routine testing as it greatly improves the throughput and allows laboratories to be more efficient. With the improvement in throughput and increasing sample complexities, analytical challenges such as carryover, contamination, high concentration positives and false positives remain a big concern.

The new MPX 2.0 software includes a new Carryover Monitoring feature that enables users to set Low and High concentration thresholds and Region Heights for various analytes in the submission method, and a logic component provides real time concentration results of target analytes. Batches of samples submitted through the Walk-Up mode software are flagged with specific messages for each sample and automatic re-injections of the sample or blank solutions can occur. This improves sample throughput by minimizing the number of sample re-injections that must be performed because of sample stream contamination or suspected carryover.

Objectives: This presentation illustrates a workflow containing compounds of forensics interest to demonstrate the usefulness of automated carryover monitoring using the MPX 2.0 software.

Methods: *Sample Preparation:* Human serum samples (VD-DDC Mass Spect Gold® MSG 1000 - Golden West Biologicals Inc.®) were spiked at a level of 1000 ng/ml for analytes of forensic interest. A 200 μL aliquot of serum was spiked with 10 μL of Internal Standard mix (20 μg/mL) in a 2 mL Eppendorf tube and vortex mixed.

Chromatography: Compounds were chromatographically separated using a Water/Methanol gradient with 10 mM Ammonium Formate and a Phenomenex 50 x 3.0 mm Kinetex® 2.6 μm Phenyl-Hexyl column.

Mass Spectrometry: The MPX system was coupled to a QTRAP® 6500+ system, which is a triple quadrupole linear ion trap hybrid mass spectrometer. Analyst 1.7 was used to acquire data and the sample batch was submitted using the walk-up feature of MPX 2.0 software. Data was processed using MultiQuant 3.0.3 software.

Results: After the batches are submitted and samples are acquired, the software reads the Quant Type and concentrations of various analytes and internal standards from the Analyst Batch information contained in the Quantitation Tab and uses the information during carryover monitoring. The software logic processes information from batch samples designated as either Unknown, Blank, or Standard. Users typically include multiple blanks as the system checks blanks for potential contamination and always ensures that a clean blank is being used.

The results include a table of sequence of events automatically triggered during a batch acquisition to demonstrate the usefulness of carryover monitoring in routine determination of compounds of forensic interest in serum samples, as well as an example of how the batch is set up.

Conclusion/Discussion: After batches are submitted during routine analysis, the software will flag samples above a certain threshold, thereby identifying those samples which may need re-analysis without the need to manually intervene and review the data. For samples showing contamination or for samples with potential contamination, the system will automatically re-inject those samples thereby reducing sample batch reruns. Following a high sample, the software will inject a series of blanks (up to three) to ensure that the streams are not contaminated, and that data quality is unaffected by carryover. By using the new software feature, laboratories can minimize sample re-injection runs and improve turnaround times significantly.

POSTER

P24: Novel Platform for Online Sample Preparation and LC-MS/MS Analysis of Drugs in Biological Matrices

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Background/Introduction: Manual sample preparation often limits the workflow capacity for today's advanced laboratory instrumentation and decrease laboratory efficiency. Having the ability to fully integrate automated sample preparation with both HPLC separation and MS detection is becoming desirable as correctly implemented automation can streamline and increase throughput as well as prevent common transcription errors. Utilizing an online automated sample preparation system coupled with an LC-MS/MS instrument demonstrates that multi-class drugs can be analyzed in postmortem biological samples with very little human intervention.

Objectives: To provide a faster, highly accurate, and reproducible approach to analyzing postmortem samples by using a fully automated sample preparation system coupled with a LCMS system.

Methods: Postmortem biological samples including whole blood, brain, and spleen were stored at 4°C. Tissue samples were homogenized in DI water at a 1:4 dilution. Samples were transferred to sample cups and placed into the CLAM-2000 (Clinical Laboratory Automated sample preparation Module). The CLAM module was connected to the LCMS and was used to automatically pretreat the samples prior to automatic transfer into the LCMS. The system was programmed to perform the sample preparation as follows: wetting filter vial with 20 µL water, sample aspiration of 10 µL, addition of 100 µL methanol/acetonitrile 1:1 (v/v), stirring, addition of 20 µL of a deuterated internal standard cocktail, second stirring, suction filtration, and finally automatic transfer of the collection tube into the LC autosampler for injection into a LC-MS/MS system. Compounds were separated using a Restek Raptor Biphenyl (100 x 2.1mm x 2.7µm) column and detected using a Shimadzu LCMS 8060 system for selected MRMs.

Results: As a proof of concept study, the methodology described provides an excellent alternative to manually preparing postmortem samples for LC-MS/MS analysis. The data displayed excellent intra-run precision as well as provided great correlation to the samples manually prepared previously. Separation was achieved using a Restek Raptor Biphenyl column for 14 target compounds. These compounds included morphine, hydromorphone, codeine, 6-acetyl morphine, hydrocodone, 7-aminoclonazepam, fentanyl, buprenorphine, lorazepam, clonazepam, nordiazepam, alprazolam, delta-9-THC, and THC-COOH. The LC-MS/MS method monitored 2 transitions for each analyte and 1 transition for each of the 4 deuterated internal standards. Total run time, including column re-equilibration, was 12 minutes. Blank blood was spiked creating a 5-point calibration curve with a linear range of 10 to 1000 ng/mL for each analyte. Each calibration curve had a linear regression value of >0.997. All curves were run in triplicate and achieved %RSDs of less than 10%. All postmortem samples which included femoral blood, heart blood, chest cavity blood, spleen, and brain were run in triplicate and commonly had a %RSD of less than 10%. When comparing the results from manual preparation of the samples to the CLAM preparation, there proved great correlation. Hydrocodone and hydromorphone, for example, exhibited <3% difference in results for manual preparation versus the automated results. The sample preparation time was reduced from ~ 2hrs when using solid phase extraction to 6.5 minutes by the automated sample preparation system.

Conclusion/Discussion: The automated sample preparation capabilities of the CLAM-2000 series coupled with the Shimadzu LCMS offers a new, hands-free approach for drug analysis in biological matrices. This automated approach increases the throughput of sample analysis by overlapping sample prep with analytical runs and allowing an analyst to perform additional tasks. The automated sample preparation approach demonstrated analysis of drugs within 10% RSD of standard manual procedures.

POSTER

P25: BAC Analysis utilizing GCMS and FID combined with Fully Automated Sample Prep performed by Robotic Sampler

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Background/Introduction: Determination of blood alcohol content (BAC) by gas chromatography-flame ionization detection (FID) or gas chromatography-mass spectrometry (GCMS) has been a standard analytical technique for many years. Though technology has advanced, many laboratories have been using the same manual sample prep procedures. Much of the GC analysis is automated; however, the process of preparing calibration standards and spiking samples with internal standards is still manually done with pipettes.

Objectives: Using robotic auto sampling reduces the measurement uncertainty associated with the analyst in the sample prep procedure while also increasing sample throughput by approximately two-fold. The ability of having the auto sampler perform dilutions and sample preparations not only removes considerable human error from the process but also provides lab technicians with more time to accomplish other lab tasks.

Methods: Robotic auto sampling was used to perform sample preparation according to a specific State Crime Laboratory requirement, by preparing the necessary dilutions for each calibrator, including the internal standard addition. The alcohol certified reference material solutions, which were used as calibrators, (0.050 g/100 mL, 0.100 g/100 mL and 0.400 g/100mL) were prepared in triplicate, and the negative control was prepared at 0.100 g/100 mL using multi-component alcohol certified reference material solution. The certified reference material consisted of methanol, ethanol, isopropanol and acetone. Also, 1.80 mL of the BAC Internal Standard Solution and 0.20 mL of the liquid were transferred into a headspace vial. The first and last sample of the sequence were both a positive and a negative control sample with any remaining required control samples distributed throughout the batch.

Results: The concentrations of ethanol, methanol, isopropanol and acetone were measured and calculated by the instrument software utilizing the most current calibration data that corresponds to the instrument used and the lot of internal standard solution used to prepare the samples. Mass spectrometry calibration curve r^2 values for methanol, ethanol, isopropanol, and acetone were recorded at 0.9996, 0.9994, 0.9998, and 0.9999 respectively. Samples run on the FID demonstrated values of 0.9999 for all components. RSD values for area were also calculated for both the GC FID and GCMS which are shown in the table below.

Compound	RSD on FID (n=6)	RSD on MS (n=6)
Methanol	1.6%	1.0%
Ethanol	1.4%	0.9%
Isopropanol	1.1%	1.5%
Acetone	0.8%	1.7%

Conclusion/Discussion: All of the samples were analyzed via GCMS and FID providing accurate qualitative and quantitative results. The required method pairing GCMS/FID as well as automated sample prep removed most human error providing a near perfect calibration curve.

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P26: Synthetic Cannabinoid Screening using High-Sensitivity QToF Mass Spectrometer and Triple Quadrupole Mass Spectrometer.

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Background/Introduction: The number of new designer drugs and their availability has exploded in recent years, leading to widespread social impact on local communities. LC-MS is used to confirm the presence of known substances involved in a particular toxicology case. However, these methods can detect only a few known substances and common designer drugs, but cannot identify new or emerging drugs of concern, like the always-evolving synthetic cannabinoids. We developed new LC-MS/MS methods using a high-sensitivity Q-TOF mass spectrometer and a fast scanning triple quadrupole mass spectrometer combined with an enhanced spectrum library to detect and screen for synthetic cannabinoids to support forensic investigations.

Objectives: To create screening methodology that can advance alongside the emerging drugs market, while maintaining accuracy and robustness of the QToF and LCMSMS.

Methods: More than 300 authentic standards for emerging synthetic cannabinoids were obtained from Cayman Chemicals (Ann Arbor, MI) and analyzed by a Q-TOF mass spectrometer and a triple quadrupole mass spectrometer (LCMSMS) to create a tandem mass spectrum library and a high-resolution tandem mass spectrum library. In order to create the libraries, product ion spectra were obtained at different fixed collision energies, and additionally the QToF acquired product ion spectra at a single collision energy of 35 eV with a collision energy spread of ±17 eV. Samples containing unknown compounds were prepared by solid phase extraction and analyzed by LC-MS/MS following centrifugation to remove particulates. Analysis was carried out using UHPLC separation, electrospray ionization, and detection in various MS modes including high resolution scan mode and data-dependent MS-MS.

Results: Accurate mass measurements of all precursor and product ions of authentic standards were within 1.5 ppm of the expected m/z, and in most cases the error was less than 0.8 ppm. Instrument resolving power was maintained above 30,000, as measured at m/z 922, over the duration of all measurements. For targeted analysis, the expected retention time was measured using authentic standards and used as part of the identification criteria. For untargeted analysis, scan mode and data-dependent MS-MS was used. Suspect peak lists were prepared by creating extracted ion chromatograms for m/z's of known formulas as well as by processing the MS data using untargeted feature extraction. Tandem mass spectra were compared to the newly created library spectra for tentative identification. Potential designer drugs in unknown samples were detected and tentatively identified using both targeted and untargeted techniques.

Conclusion/Discussion: The QToF mass spectrometer and the triple quadrupole mass spectrometer are powerful screening tools for the forensic community to use in order to stay on top of the emerging drugs on the market.

POSTER

P27: Evaluation of a Prototype Device for Rapid Forensic Drug Screening

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Background/Introduction: In recent years there has a been an increase in the number of deaths associated with common club drugs such as cocaine, ecstasy (MDMA) and ketamine. This worrying trend has been attributed to a dramatic rise in the availability of 'super-strength' pills and high purity drugs. In addition, the last decade has seen the emergence of more than 800 new psycho-active substances and synthetic analogues for which there is often little, to no, toxicity data for the individual agents, and even less information when used in combination with other substances. The increase in number, diversity and potential toxicity of drugs is a major concern for police authorities and health care teams alike; it also presents significant challenges for the laboratories who are involved in the analysis of seized substances. Consequently, methods that can facilitate the rapid screening of drugs, are of interest.

Objectives: The aim of this study was to evaluate the performance of a prototype device based on Atmospheric pressure Solids Analysis Probe-Mass Spectrometry (ASAP-MS) for rapid drug screening and to compare data with an established screening method based on high-resolution mass spectrometry (HRMS).

Methods: A small-footprint prototype device incorporating ASAP-MS was used for these experiments. Samples (certified reference material, pills, powders, resin) were analysed following either an initial dilution with methanol and subsequent spotting of 2 µL aliquots onto a glass capillary tube, or after 'dipping' the capillary tube into the crushed sample. Following insertion of the capillary into the device, mass detection was performed using full scan *m/z* 60-650.

The results from the nominal mass prototype device were compared with an established HRMS method *i.e.* UNIFI™ Forensic Toxicology Screening Solution (Waters) comprising an ACQUITY UPLC I-Class in combination with a Xevo G2-XS QTOF. This reference method comprised a 15 min chromatographic separation and data were acquired using MS^E which facilitates collection of data at low and high collision energies providing accurate mass for precursor and diagnostic fragment ions. HRMS data was compared with a library comprising >1600 toxicologically-relevant substances. Identification was based on retention time (± 0.35 min of reference), precursor mass and at least one fragment ion within 5 ppm of exact mass.

Results: The prototype device enabled direct analysis of drug substances; a heated desolvation gas was used to volatilize the sample and a corona discharge to ionize. To improve specificity, in-source collision-induced dissociation (CID) was performed and four differing cone voltages were selected: 15, 30, 50 and 70V, which resulted in the generation of both precursor and product ions for improved drug identification.

The study assessed various samples including 40 certified reference material (CRM), 20 pharmaceuticals, 10 natural supplements/herbal medications and more than 60 unknown samples that had been confiscated at various music events/venues by the local police.

A small library was generated from ASAP-MS of the CRM; analysis of the other preparations *e.g.,* pharmaceutical and seized samples, demonstrated very good qualitative agreement when compared with this library. Analysis of an aliquot of methanolic solutions was considered more consistent than 'dipping' *i.e.,* direct analysis of crushed material. For the seized samples, ASAP-MS indicated that 40% of the seized samples contained ketamine, 30% contained MDMA and 20% contained cocaine. Other drugs identified were mixtures of these three drugs or MDMA/MDEA, paracetamol, sildenafil. Confirmatory analysis by HRMS showed excellent agreement (> 95%) with the major components identified by ASAP-MS. HRMS also revealed presence of additional lower concentration adulterants/impurities.

Conclusion/Discussion: The prototype device was very easy to use and very promising for a rapid identification of drug substances. Results were obtained within 2 min and showed very good qualitative agreement with a comprehensive HRMS screening method.

POSTER

P28: Fentanyl Metabolites and Precursors in Postmortem Blood Compared with Plasma following Surgical Administration of Fentanyl

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Introduction: Analytical methods for detection and quantitation of 4-anilino-N-phenethyl-4-piperidine (4-ANPP), fentanyl and fentalogs (fentanyl analogs or metabolites) continue to be developed and applied in postmortem toxicology practice to assist in the determination of cause and manner of death. In fentanyl related fatalities, co-detection of 4-ANPP in casework may be due to either inefficient illicit-synthesis or metabolism. In addition, co-detection of fentanyl with fentalogs including β-hydroxyfentanyl and acetyl fentanyl may be due to fentanyl metabolism rather than co-administration of an illicit agent. Measurement of these compounds following administration of pharmaceutical-grade fentanyl may provide insight into illicit synthesis versus metabolite production in postmortem casework.

Objectives: The objective of this work was to compare results of analysis of 4-ANPP, fentanyl and fentalogs in postmortem blood from a series of fentanyl and fentalog related fatalities with those obtained following analysis of discarded plasma from patients receiving intravenous administration of pharmaceutical-grade fentanyl.

Methods: De-identified blood was obtained from 44 fentanyl related fatalities. Postmortem blood was analyzed using UPLC-MS/MS. Analytes included fentanyl, 4-ANPP, norfentanyl, and other fentalogs *e.g.,* acetyl fentanyl, β-hydroxyfentanyl, furanyl fentanyl, *etc.* Chromatographic separation was achieved using a Waters ACQUITY BEH C_{18} column after an initial precipitation step, followed by clean-up using a Waters Oasis PRiME MCX µElution plate.

Discarded serial plasma samples (70) were obtained from 18 surgeries where pharmaceutical-grade fentanyl was administered intravenously. Subjects received 50-150 µg of fentanyl upon induction of anesthesia with further administration as needed. Plasma was analyzed for 4-ANPP, fentanyl and the fentalogs by UPLC-MS/MS using a Waters ACQUITY CSH C_{18} column and direct sample clean-up on a Waters Oasis PRiME MCX µElution plate. Institutional Review Board approval was obtained for the study through the Albany Medical College. The analysis methods were validated for recovery, matrix effects, linearity, accuracy, precision, sensitivity and carryover.

Results: In the postmortem study, the frequency of detected analytes was as follows: 4-ANPP (44), fentanyl (36), norfentanyl (35), β-hydroxyfentanyl (29), acetyl fentanyl (11), fluoroisobutyryl fentanyl (9), methoxyacetyl fentanyl (5), furanyl fentanyl (5) and cyclopropyl fentanyl (3). Norfentanyl and β-hydroxyfentanyl were associated with 97% and 81% of 36 fentanyl-related fatalities, respectively. In the 9 postmortem cases without detectable fentanyl, neither norfentanyl or β-hydroxyfentanyl were present. The median concentrations and ranges for fentanyl (11.0 ng/mL; 0.21- >20), 4-ANPP (0.39 ng/mL; 0.1->20), norfentanyl (1.35 ng/mL; 0.05-13.6) and β-hydroxyfentanyl (0.27 ng/mL; 0.05-2.6) were determined, with β-hydroxyfentanyl averaging 16% of the norfentanyl concentration.

In parathyroidectomy cases, the 16 pre-administration plasma samples were negative for all analytes. In the specimens containing fentanyl, 4-ANPP was not measurable above the assay's quantitation limit of 0.005 ng/mL and acetyl fentanyl was only detected in one specimen. Norfentanyl and β-hydroxyfentanyl were detected in association with fentanyl in 100% and 48% of the post-administration plasma, respectively. However, many of the "negative" β-hydroxyfentanyl samples appeared to have detectable compound below the LLOQ (0.005 ng/mL). The median concentrations and ranges for fentanyl (0.34 ng/mL; BDL-1.5) norfentanyl (0.017 ng/mL; BDL-0.045) and β-hydroxyfentanyl (0.008 ng/mL; BDL-0.020) were determined with β-hydroxyfentanyl averaging 43% of the norfentanyl concentration.

Conclusion/Discussion: The concentration of 4-ANPP and fentanyl overlapped in the postmortem study, but 4-ANPP was undetected following administration of IV fentanyl during surgery, suggesting that metabolism is not a major source of the 4-ANPP detected in casework, despite the lower doses used in the surgical study. The association of β-hydroxyfentanyl with fentanyl and norfentanyl in both the postmortem and surgical studies indicates metabolism of fentanyl to β-hydroxyfentanyl. Use of 4-ANPP as a marker of illicit fentanyl production and β-hydroxyfentanyl as an additional metabolite marker of fentanyl use may assist in advancing the interpretation of forensic casework.

P29: Incidence and Concentrations of Amphetamine and Methamphetamine in Whole Blood Samples Obtained in Drugs and Driving Cases (OUI) in the Commonwealth of Massachusetts

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Background/Introduction: In this presentation, attendees will learn about the extraction and analysis method for Amphetamine (AMP) and Methamphetamine (MAMP) from the whole blood samples in drug/driving and cases employing commercially available solid phase extraction (SPE) cartridges and analysis of the extracts using Liquid Chromatography-Mass Spectrometry (LC-MS/MS).

At therapeutic doses, AMP/MAMP cause emotional and cognitive effects such as euphoria, change in desire for sex, increased wakefulness, and improved cognitive control. These compounds may induce physical effects such as decreased reaction time, fatigue resistance, and increased muscle strength. In the case of operating motor vehicles even therapeutic doses may impair the ability of the driver to control the vehicle safely.

Objectives: This project was initiated with the idea of developing a simple, robust SPE method for the extraction and LC-MS/MS analysis of AMP/MAMP in Drugs and Driving cases. The data would be collected to assist the submitting enforcement agencies with interpretation by comparing blood concentrations with impairment details.

Methods: Aliquots (1.0 mL) of whole blood (calibrators, controls, and test samples each containing deuterated AMP/MAMP internal standards) were diluted with 3.0 mL of aqueous phosphate buffer (0.1 mol/L, pH 6) and vortex mixed and centrifuged for 10 minutes at 3000 rpm. The supernatant liquid was applied to mixed mode strong cation exchange SPE columns pre-conditioned with methanol, deionized water (DI H_2O) and phosphate buffer (3 mL each, respectively). The SPE columns were washed with DI H_2O, aqueous acetic acid, methanol (3 mL, respectively), dried for 10 minutes under full flow using a positive pressure SPE manifold and nitrogen as the drying gas. Each SPE column was eluted with 1 x 3 mL of a solution consisting of dichloromethane-propan-2-ol-ammonium hydroxide (78-20-2) and the eluates were collected in glass sample tubes. 100 μL of LC-MS/MS mobile phase was added to each eluate, vortex mixed and were evaporated to approximately 100μL under a gentle stream of nitrogen at 35 ºC. To this solution was added an additional 100 μL of LC-MS/MS mobile phase and vortex mixed before being transferred to individual auto sampler vials for analysis LC-MS/MS was performed on 50mm x 2.1mm (5 μm) polyaromatic LC phase column coupled to a guard column of same chemistry using a mobile phase consisting of acetonitrile containing 0.1% formic acid/aqueous 0.1 formic acid. The flowrate was set at 0.5 mL/minute. Mass spectrometry was carried out in positive multiple reaction mode ((MRM). Analytical details presented.

Results: The limits of detection/ quantification for this method for AMP/MAMP were determined to be 5.0 ng/mL and 10 ng/mL, respectively for the analytes. The AMP/MAMP method was found to be linear from 10 ng/mL to 1000 ng/mL (r^2>0.999). The AMP/MAMP recoveries were found to be greater than 95%. Interday and Intraday variation of method were found to < 7% and < 9 %, respectively. Matrix effects were determined to be < 5% for AMP/MAMP SPE method. Details regarding the concentrations of AMP/MAMP found in genuine drugs and driving cases ranged from: 174 Males: median age: 28 years old (AMP: 15ng/ mL to 506 ng/ mL: median=378ng/ mL), (MAMP: 34 ng/ mL to 882 ng/ mL: median= 620ng/ mL); 82 Females: median age: 24 years old (AMP:12 ng/ mL to 472 ng/ mL: median= 288ng/ mL), (MAMP: 28ng/ mL to 724ng/mL: median = 545ng/ mL).

Conclusion/Discussion: This study compares the incidence and concentrations of AMP/MAMP found in whole blood samples of drivers suspected of driving under the influence of drugs including AMP/MAMP. These types of samples are frequently presented to forensic toxicology laboratories for analysis and interpretation. Previous methods of analysis of these compounds has employed GC-MS after derivatization, whereas the current method eliminates this time-consuming step. It also removes the need to evaporate and re-constitute eluates by using the mobile phase as a keeper solvent.The data show that AMP is a more frequently observed drug than MAMP in blood samples taken in the Commonwealth of Massachusetts obtained in drugs and driving cases. It must be noted that AMP is a metabolite of MAMP and both compounds are psychoactive.

POSTER

P30: Was It Worth It: The Pros and Cons of Implementing a Full DUID Testing Policy

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Background/Introduction: Two years ago, the Henderson Forensic Laboratory made a major change to the testing policy for the DUI/DUID cases submitted to the lab. The Henderson Forensic Laboratory's toxicology section went from testing only DUI cases with a blood alcohol concentration (BAC) below the per se level plus the uncertainty of measurement (0.084G%) for drugs in the blood to testing all DUI samples for alcohol and drugs regardless of the blood alcohol concentration. Any change to a single testing policy can be difficult. When the change involves the entire testing process then it is inevitable that you will run into numerous challenges and obstacles. However, if the correct policies are put into place to make the process as smooth, efficient and cost effective as possible, then the results that you can achieve and the data that you are able to collect and pass onto others can be substantial and extremely beneficial.

Objectives: To discuss the challenges and accomplishments of implementing a full DUI/DUID testing policy in the current forensic toxicology laboratory as well as discuss the findings that have been attained so far.

Methods: In order for this new testing policy to be successful at the Henderson Forensic Laboratory there had to be specific testing procedures in place that would make the new policy more efficient, more robust, less expensive and as timely as possible. Under the old policy, DUI whole blood samples would first be analyzed for ethanol and other volatile compounds. Any ethanol concentrations below 0.084G% would be sent on for further toxicology testing for drugs or other impairing compounds. On average this consisted of sending approximately 25% of all DUI cases onto further testing. The other 75% of cases were reported out and testing was completed. In order to go from doing drug screening and confirmation testing on 25% of all DUI samples to 100% of all DUI samples, the forensic laboratory had to change the entire workflow from the volatiles analysis through the confirmation testing of each drug. Changes were made to the drug screening and drug confirmation process to get away from lengthy extractions, extensive data processing methods and expensive testing to more streamlined extractions, automated data processing and more financially feasible testing by moving away from lengthy ELISA and GC/MS methods.

Results: During the past two years there have been approximately 1273 DUI cases submitted to the Henderson Forensic Laboratory's toxicology section. These samples come from four different agencies in southern Nevada that service a population of approximately 600,000 citizens. Of the 1273 cases, 369 (29%) of the cases had a BAC of 0.084G% or below and would have been sent onto further testing under the old policy. The other 904 cases (71%) would have been reported out with only the blood alcohol results. For the 904 cases with a BAC above 0.084G%, 598 (66%) had drugs in their system and 306 (34%) were drug free and only had ethanol in their blood sample. During this two-year time period the five most prominent drugs seen in cases with a BAC above 0.084G% were (in order): Marijuana, Alprazolam, Methamphetamine, Amphetamine, and Carisoprodol.

Conclusion/Discussion: Since the legalization of recreational marijuana in Nevada in 2017 there has been a big push to attain data on drug impaired driving in the state. As a result of this, a bill was introduced in the Nevada legislature this year (Nevada SB 23) that would legislate that all publicly funded forensic toxicology laboratories in the state of Nevada adopt testing policies similar to that of the Henderson Forensic Laboratory and perform alcohol and drug testing on all DUI samples.

P31: Difluoroethane Exposure

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Background/Introduction: 1,1-Difluoroethane (DFE, HFC-152a) is a hydrofluorocarbon commonly used as a propellant in air duster products and is also a refrigerant, although it is not commonly used for that purpose. DFE is a volatile compound that can cause central nervous system (CNS) depression and related impairment to human performance. In a series of 16 impaired driving case reports involving DFE 81% involved a traffic crash and impairing effects noted included confusion, lethargy, impaired judgement, loss of motor coordination, and loss of consciousness (1).

Limited studies have been published describing the pharmacokinetics of DFE, however they indicate a short window of detection (2). In a controlled study of occupational exposure to DFE in humans the average peak concentrations reached were only 2.3 µg/mL after two hours of sustained exposure (2). Elimination was in two phases: during the initial rapid phase concentrations dropped to below 0.7 µg/mL after ~12 minutes. During the slower phase the concentration dropped to below ~0.0007 µg/mL by 22 hours (2). DFE has been detected in impaired driving cases up to 2.5 hours after the crash or traffic stop, at or above ~2.6 µg/mL (1).

Objective: To study the plausibility of detecting DFE in blood (above ~2.6 µg/mL) several hours after an extreme occupational exposure scenario.

Method: A 40 year-old, 150 lb, male volunteer discharged air duster products containing DFE while inside a confined space of a 2006 Ford F-150 Extended Cab pickup truck. Two canisters were discharged in an alternating fashion using short bursts for a period of 15 minutes until the canisters became inoperable due to freezing over. Canister 1 was a 10 oz. (283g) net weight Dust-Off brand air duster. Canister 2 was a 7 oz. (198g) net weight Dust-Off brand air duster.

A whole blood specimen was collected ~3 hours and 20 minutes after exposure. Urine specimens were collected at ~85 minutes and ~4 hours after exposure. The blood and urine specimens were analyzed using a previously described method with a limit of detection of ~2.6 µg/mL (7).

Results: Approximately 162g (57%) in canister 1 and 113g (57%) in canister 2 was used during the 15 minutes exposure.

DFE was not identified in the blood specimen or either urine specimen collected. The urine specimen collected 85 minutes after exposure did show an indication of the presence of DFE, but with a weak response that was below the limit of detection of the method and could not be identified.

Conclusion/Discussion: DFE is a central nervous system depressant that, when abused, can cause impairment to faculties required for the safe operation of a motor vehicle. Driving cases in which it is detected often involve collisions (1). Occupational exposure, even under extreme conditions, did not result in detectable levels (above ~2.6 µg/mL) in blood collected 3.3 hours after exposure or urine collected 1.4 and 4 hours after exposure.

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POSTER

P32: An Analysis of the Relationship between DUI Arrests, Fatality Crashes, and Missouri v. McNeely in Harris County, Texas

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Introduction: Harris County is the 3rd largest county in United States with a population of 4.7 million people. We studied the fatality crashes and DUI arrests before and after the implementation of the McNeely law. In Schmerber vs. California (1966), the Court allowed a police officer to collect blood evidence without a warrant under the exigent circumstances exception to prevent the destruction of alcohol through the body's natural metabolic processes. Later, Missouri vs. McNeely (2013) changed the requirement, stating that police must generally obtain a warrant before subjecting an impaired driving suspect to a blood test, and that the natural metabolism of blood alcohol does not establish a per se exigency that would justify a blood draw without a warrant. The Court left open the possibility "exigent circumstances" might apply in some impairment driving cases. However, the District Attorney in Harris County, Texas issued a mandate after the McNeely ruling requiring a search warrant when collecting blood evidence.

Objectives: This presentation analyzes the McNeely ruling and attempts to determine if this ruling has impacted impairment arrests and fatality crashes. The study is guided by the following research questions: (1) Is there a significant difference in DUI arrests after the Missouri v. McNeely ruling? (2) Is there a significant difference in fatality crashes after the Missouri v. McNeely ruling?

Methods: A review of arrests and fatality crashes from 2007 through 2016 was conducted. A total of 121,814 impairment arrests were made and 3,542 fatality crashes occurred in Harris County, Texas. The Analysis of variance (ANOVA) is used to analyze the differences between arrests and fatalities crashes in Harris County after McNeely. The one-way ANOVA was used to compare the mean. The Tukey test identified where the difference occurred. The content analysis reviewed the exigent circumstances policy from the Harris County District Attorney's Office.

Results: In the observed data for arrests, the analysis revealed Harris County had a 42% decrease in impairment arrests between 2011 (highest arrest year) and 2014 (lowest arrest year) with P-value: 1.75506. In the observed data for fatalities, the analysis revealed Harris County had a 32% increase in fatality crashes between 2009 (lowest fatality crash year) to 2016 (highest fatality crash year) with P-value: 1.5791. The analysis shows that there was a significant difference between the means, which indicates the outcome was unlikely to have occurred by chance. Therefore, the null hypothesis is rejected. There is sufficient evidence (test statistically is within the rejection region) to conclude the McNeely ruling did decrease DWI arrests in Harris, County, Texas. Harris County had a decrease of 3% over the ten-year time period.

Conclusion/Discussion: The design of data collected for this study has a limitation: length of time evaluated. Over the ten-year period technology for gathering data improved. However, impairment arrests declined in Harris County, Texas after it became mandatory to obtain a warrant for blood evidence. This study utilized data over a ten-year period. Therefore, population growth and how it affects criminal behavior should be considered in future research.

P33: Validation of a 14 Assay ELISA Panel for Tier 1 Recommended Compounds in DUID Investigations

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Background/Introduction: The published recommendations for Tier 1 testing in Driving Under the Influence of Drugs (DUID) investigations[1] are designed to be achievable with immunoassay and/or chromatographic screening techniques. The 2017 update added fentanyl, buprenorphine, and tramadol to Tier 1, moved barbiturates and phencyclidine (PCP) to Tier 2, and grouped benzodiazepines into low dose and high dose categories. Laboratories may need to update their testing schemes in order to meet these guidelines.

Objectives: To develop and validate an Enzyme Linked Immunosorbent Assay (ELISA) panel for screening blood and urine specimens in DUID investigations. The assays and decision points were selected to achieve compliance with published guidelines for Tier 1 compounds, with the addition of PCP due to prevalence in current casework. The method validation requirements were based on the proposed ASB Standard 036 Standard Practices for Method Validation in Forensic Toxicology (draft released for public comment in September 2018) rather than the published SWGTOX guideline, due to the proposed updates for ELISA validation.

Methods: The following assays were validated in blood and urine: amphetamine, benzodiazepines, benzoylecgonine, buprenorphine, cannabinoids, carisoprodol, fentanyl, methadone, methamphetamine, opiates, oxycodone, phencyclidine, tramadol, and zolpidem.

A Dynex DSX instrument was used for the analysis. During method development, calibrators, dilutions, and sample volumes were adjusted to obtain a decision point B/B0 around 50% and achieve as much differentiation around the decision point as possible. Kits from Immunalysis and Neogen were evaluated in development and resulted in a mix of both manufacturers being selected for validation.

Precision studies were conducted in accordance with the draft standard. Limit of Detection (LOD) was assessed mathematically for kits used in accordance with the manufacturer's recommended calibrator and dose (pg/well). LOD was assessed experimentally for all other assays for compounds having lower cross reactivity than the calibrator. Carryover was evaluated at 50x the decision point concentration. Processed sample stability was evaluated over two days. The cross reactivity of l-amphetamine and l-methamphetamine was evaluated on the amphetamine and methamphetamine kits, respectively.

Results: All assays for both matrices passed the 20% CV precision requirement. For blood, all assays passed the 2SD requirement except carisoprodol, methamphetamine, and opiates. In urine, only the benzoylecgonine assay failed the 2SD requirement. In all assays which failed this requirement, the -50% cutoff was the concentration that failed, with results slightly out of the acceptable range.

The LOD results show compliance with the screening cutoff recommendations for Tier 1 compounds of the DUID recommendations, except for hydromorphone and 7-aminoclonazepam. These two compounds will not screen positive unless concentrations exceed the recommended cutoffs. Hydromorphone demonstrated cross reactivity of 67% in blood and 49% in urine, while 7-aminoclonazepam showed 48% in blood and 47% in urine. These values do not meet the 80% cross reactivity recommendation. The l-amphetamine and l-methamphetamine exhibited negligible cross reactivity when evaluated on the amphetamine and methamphetamine kits, respectively.

The carryover experiment resulted in no observed carryover in blood. However, carryover was observed in urine for carisoprodol at 25,000 ng/mL, methadone at 15,000 ng/mL, and methamphetamine at 10,000 ng/mL.

All assays for both matrices passed the stability study.

Conclusion/Discussion: While the validation did not meet all criteria in the ASB draft standard, it was determined that the fourteen (14) assay ELISA panel is valid and fit for purpose. Intra-day data were acceptable, however for three blood assays and one urine assay the inter-day data slightly exceeded the 2SD requirement. Extensive method development achieved compliance for all the Tier 1 recommended compounds, except 7-aminoclonazepam and hydromorphone. This study demonstrates that ELISA is a valid screening method for DUID investigations.

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POSTER

P34: Optimization of the Neogen® Benzodiazepine ELISA Kit for DUID Investigations

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Background/Introduction: Screening for benzodiazepines by immunoassay can be challenging due to the variable cross reactivities (XR) and wide range of doses encountered within this drug category. Manufacturers typically use a high dose benzodiazepine, such as oxazepam or nordiazepam, as the calibrator. This proves to be ineffective at detecting relevant levels of low dose benzodiazepines. Some high prevalence benzodiazepines, like clonazepam, often have low XR in commercially available kits.

Published recommendations for Driving Under the Influence of Drugs (DUID) investigations[1] require the ability to detect low dose benzodiazepines at 10 ng/mL and 50 ng/mL in blood and urine respectively, along with high dose benzodiazepines at 50 ng/mL and 100 ng/mL respectively. If using immunoassay screening, target compounds should have at least 80% XR to the target compound. Given these requirements for multiple detection limits and a minimum XR level within a single immunoassay screen, careful planning and data evaluation is needed during method development.

Objectives: To develop an Enzyme-Linked Immunosorbent Assay (ELISA) protocol for the screening of benzodiazepines in blood and urine that would meet published recommendations for Tier 1 benzodiazepines.

To evaluate the XR of all benzodiazepine compounds listed within the scope of the DUID guidelines. These scope compounds include alprazolam, alpha-hydroxyalprazolam, clonazepam, 7-aminoclonazepam, lorazepam, diazepam, nordiazepam, oxazepam, and temazepam. Confirmation methods also include midazolam and etizolam, so their XR was also evaluated during this study.

Methods: Dynex DSX instruments with full automation were used. Kits from Immunalysis and Neogen were evaluated in development, and ultimately the Neogen kit was chosen due to improvements in precision and discrimination between concentration points. Lorazepam was chosen as the target analyte for improved sensitivity and XR with other scope benzodiazepines. The lorazepam cutoff concentration was set at 10 ng/mL in the blood assay, with a 100 µL sample volume and 1:10 sample dilution. The urine assay cutoff was set at 40 ng/mL, with a 50 µL sample volume and 1:20 sample dilution.

A B/B_0 versus concentration curve was established for each analyte. Negative and lorazepam cutoff controls were analyzed concurrently with each curve. The limit of detection (LOD) was established by determining the concentration of the analyte that would exhibit a B/B_0 response equal to that of the lorazepam cutoff. The analyte XR was then estimated from that LOD value as %XR = Calibrator Concentration (ng/mL)/LOD Concentration (ng/mL)*100.

Results: Experimentally determined XR values were >80% for all compounds, except for 7-aminoclonazepam (~47% for both matrices) and midazolam (62.5% in blood; 76.9% in urine). Experimentally determined XR values for many compounds were significantly different than those reported by the manufacturer[2]. These unexpected results were counterintuitive based on how XR was expected to shift when using lorazepam (70% XR relative to oxazepam) as the target analyte.

Conclusion/Discussion: The Neogen Benzodiazepine ELISA kit was determined to be fit for purpose as a screening method for DUID investigations. With the exception of 7-aminoclonazepam, study results were compliant with current Tier 1 DUID recommendations. The discrepancies in XR values from those reported by the manufacturer could be attributed to the fact that the manufacturer performed XR studies in buffer, rather than in matrix, or differences in assay cutoffs, sample volumes, and/or sample dilutions. This highlights the importance of in-house XR studies to accurately determine the detection capabilities of an immunoassay screen.

1. Logan, B.K., D'Orazio, A.L., Mohr A.L.A., Limoges, J.F., Miles, A.K., Scarneo, C.E., Kerrigan, S., Liddicoat, L.J., Scott, K.S., Huestis, M.A. (2018) Recommendations for Toxicological Investigation of Drug-Impaired Driving and Motor Vehicle Fatalities-2017 Update. *Journal of Analytical Toxicology*, **42(2)**, 63-68.

2. Neogen Benzodiazepine Group (Oxazepam/Clonazepam) ELISA Kit Insert

P35: Analysis of CBN, CBD, and CBG in Blood and Oral Fluid DUI/D Cases

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Background/Introduction: Although classified by the DEA as a Schedule I drug, medical and recreational marijuana have been legalized in 33 and 10 states as well as the District of Columbia, respectively. As a result of this push towards decriminalization, more focus has been placed on testing for marijuana use and its effects on driving. Detection of delta-9-tetrahydrocannabinol (THC), the primary psychoactive component in marijuana, along with other cannabinoids such as cannabinol (CBN), cannabidiol (CBD), and cannabigerol (CBG) have been explored as markers for recent use of marijuana.

Objectives: To investigate the prevalence of THC, its metabolites, and CBN, CBD, and CBG in blood and oral fluid (OF) cases analyzed by the Alabama Department of Forensic Sciences.

Methods: Blood samples from driving under the influence (DUI/D), traffic crashes, and other case types were screened by enzyme immunoassay using either a Tecan Freedom Evo75 with Immunalysis reagents or a Randox Evidence Analyzer. OF specimens were collected in select DUI cases using Quantisal® collection devices. Confirmation and quantification of THC, its two metabolites, 11-nor-9-carboxy-delta-9-THC and 11-hydroxy-delta-9-THC, and CBN, CBD, and CBG were performed by liquid-liquid extraction followed by analysis using an Agilent 6460 Triple Quadrupole LC/MS/MS. An Agilent Poroshell 120 EC-C18, 2.1x100, 2.7 micron column was used along with mobile phase A/B of 0.1% formic acid in water/methanol. LOD and LLOQ for 11-nor-9-carboxy-delta-9-THC was 2.5 ng/mL and 5.0 ng/mL respectively. All other targets had a LOD and LLOQ of 0.5 ng/mL and 1.0 ng/mL.

Results: Testing for CBN, CBD, and CBG in blood samples was added to the procedure in March 2019. Since the inclusion of these other cannabinoids, 217 cases that screened positive for cannabinoids by immunoassay have been analyzed using this method and 211 cases were confirmed as having at least one cannabinoid (97%) . 197 cases contained THC (91%), 60 cases contained CBG (28%), 22 contained CBN (10%), and 11 contained CBD (5.1%). The median concentrations for all targets are listed below separated by case type. In six DUI cases, OF specimens were collected along with the blood. Five of these OF samples were positive for THC (83%), five were positive for CBG (83%), four were positive for CBN (67%), and COOH-THC, OH-THC, and CBD were not detected.

Table 1 – Cannabinoid Concentrations in Whole Blood

Case Type	Median THC (ng/mL)	COOH-THC (ng/mL)	Median OH-THC (ng/mL	Median CBG (ng/mL)	Median CBN (ng/mL)	Median CBD (ng/mL)
DUI/D (n=102)	3.9	33	2.5	1.1	<1.0	<1.0
Traffic Crash (living) (n= 23)	4.3	34	3.2	<1.0	1.7	ND
Traffic Crash (deceased) (n=18)	10	52	3.2	1.4	2.3	<1.0
Other case types (n=68)	9.5	27	3.0	1.4	1.5	<1.0

Conclusion/Discussion: It has been previously shown by Huestis et al, that cannabinoids such as CBG and CBN are markers of recent use. The addition of testing for CBN, CBD, and CBG along with THC and its metabolites, construct a more complete representation of recency of marijuana use. This should be coupled with behavioral/impairment observations and crash risk data when providing interpretation of the results. Comprehensive monitoring of cannabinoid prevalence and blood concentrations in traffic arrests, crashes, and fatalities provides valuable information for many entities such as NHTSA and FARS. The higher prevalence of CBG and CBN in oral fluid than blood highlights one of the advantages of oral fluid drug testing in DUI/D cases which is the ability to collect a sample close to the incident of driving (e.g. at the roadside).

P36: A Qualitative Method for an Expanded Benzodiazepine Panel in Blood and Urine Using LC-QTOF

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Background/Introduction: The expanding variety of drugs potentially involved in drug facilitated crimes necessitates methodologies to match. While the majority of cases involving benzodiazepine class drugs contain a relatively small variety of these drugs, some cases contain compounds beyond "conventional" benzodiazepines that may go undetected. For laboratories with large drug panels, achieving low limits of detection can be challenging, especially in drug facilitated crime case samples. By refining the laboratory's methodologies with its existing instrumentation, this method produces an expanded confirmatory panel for benzodiazepines and Z-drugs, incorporating newer and less frequently seen compounds in blood and urine matrices such as pyrazolam, adinazolam, cinolazepam and diclazepam. This was achieved without increasing the costs or time required to process each sample and has been used to detect compounds in cases that our previous methods would have failed to identify.

Objectives: The objective of this project was to develop a confirmatory method with low limits of detection for an expanded library of benzodiazepine class drugs in blood and urine using liquid chromatography-time-of-flight mass spectrometry (LC-QTOF) suitable for detection of drugs in human performance toxicological testing. This paper also discusses the application of this methodology and presents one case of a sexual assault victim.

Methods: Sample preparation: 1 mL of blood was fortified with an internal standard mix were diluted with 2 mL of pH 7.4 phosphate buffer and incubated at RT for 15 min. The samples were centrifuged at -10°C, 3500 rpm for 10 min. The supernatant was transferred to Oasis HLB extraction cartridges and washed with water. An extraction rinse solution comprised of 40:60:0.5 (v/v/v) methanol: water: ammonia. The columns were eluted with an 80:20 (v/v) dichloromethane: isopropanol solution. The eluents were evaporated using nitrogen gas flow at 9 psi at 35°C. The extracts were then reconstituted in 50 μL of aqueous mobile phase (70:30 ((v/v)) 5 mM ammonium formate, 0.1% formic acid in water and 0.1% formic acid in acetonitrile). Instrumental analysis: The extracts were separated using an Agilent 1260 HPLC fitted with an Agilent Poroshell 120 EC, 2.7 μM, 2.1 x 100 mm column and subsequently analyzed on an Agilent 6530 QTOF system operated in TargetMS2 acquisition mode with a collision energy of 20 eV. The results were processed using MassHunter software and a database built by our lab. Method validation was performed in accordance with SWGTOX guidelines and included the parameters: limit of detection, matrix effects, stability, carryover, interference, and ion enhancement and suppression.

Results: Thirty-nine benzodiazepine and 3 Z-drug compounds were validated at LODs of 5 ng/mL or lower, and as low as 1 ng/mL for most compounds. No carryover was observed up to 1000 ng/mL. There were no observable endogenous or exogenous interferences when examining 10 matrices and over 100 therapeutic and illicit drugs. Extracts were stable for at least 24 hours after being reconstituted. Ionization suppression was observed for several compounds which had an effect on the assay's limits of detection. That said, all drugs were detected at 5 ng/mL across 10 matrices from multiple sources.

Conclusion/Discussion: The method validated and discussed here proved to be sensitive, free from interference, and included many of the new designer benzodiazepines. Although ionization suppression was observed for some of the drugs within this target panel, sensitivity down to 5 ng/mL was demonstrated by all compounds across several matrices. This satisfies all Drug Facilitated Crime Committee guidelines.

P37: A Comparison of Current Methodologies for Extraction of Drugs of Abuse from Urine

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Background/Introduction: Urine is a very useful matrix in forensic laboratories because it is easy to collect and can help determine if a drug has been used recently. There are many viable sample preparation options for urine samples, which include phospholipid depletion, supported liquid extraction, solid phase extraction, and more novel technologies, like dual mode extraction. These sample preparation techniques each offer different levels of cleanliness and analyte recovery. Results from a drugs of abuse panel with 100 compounds in urine was compared by LC-MS/MS using the different extraction techniques and popular hydrolysis procedures, to identify practical considerations during method development.

Objectives: The objective of this study was to determine the urine clean-up method that resulted in the cleanest samples and highest analyte recovery using standard hydrolysis protocols followed by LC-MS/MS analysis.

Methods: A 100 μL urine sample was used for each sample preparation technique. The samples were hydrolyzed with one of four hydrolysis enzymes to determine which yielded maximum hydrolysis efficiency (Kura BG100, Kura BGTurbo, IMCSzyme, and Campbell Science Abalone). After hydrolysis, the samples were extracted. For the phospholipid depletion protocol (ISOLUTE PLD+, Biotage), acetonitrile or acetonitrile with 0.1% formic acid was used. For the dual mode extraction (ISOLUTE DME+, Biotage), acetonitrile or acetonitrile with 0.1% formic acid was used. For the supported liquid extraction (ISOLUTE SLE+, Biotage), an elution solvent of 95:5 dichloromethane/isopropanol was used. For the solid phase extraction (EVOLUTE EXPRESS CX, Biotage), an elution solvent of 78:20:2 dichloromethane/methanol/ammonium hydroxide was used. Following extraction, the samples were evaporated and reconstituted in a 90:10 mix of 0.1% formic acid in water/0.1% formic acid in methanol and analyzed using a Shimadzu Nexera X2 UPLC coupled with a Sciex 5500 tandem mass spectrometer.

Results: Recoveries and matrix effects were calculated for all compounds for each extraction technique using each enzyme. The supported liquid extraction and solid phase extraction methods resulted in the cleanest extracts (lowest matrix effects). Recovery varied greatly for each compound depending on the extraction technique used. Some compounds, like gabapentin and pregabalin, had recoveries of 100% when using EVOLUTE EXPRESS CX and had recoveries of less than 10% when using other techniques. Other compounds (opioids and benzodiazepines) had recoveries of at least 80% for all extraction techniques. The fastest extractions were performed using ISOLUTE PLD+ and ISOLUTE DME+. The solid phase extraction method using EVOLUTE EXPRESS CX was the extraction technique that took the most time for completion. The four hydrolysis enzymes that were tested did not result in significant variation in recoveries or matrix effects (most compounds had recovery and matrix effect calculations within 20% of each other for all enzymes tested). This indicates that the extraction techniques were able to eliminate the hydrolysis enzymes from the eluted samples.

Conclusion/Discussion: When analyzing a 100 compound drugs of abuse panel in urine, it is important to look at sample cleanliness and analyte recovery in order to select the optimum method. Phospholipid depletion and dual mode extraction have higher recoveries for some analytes of interest but they also have increased matrix effects. Supported liquid extraction and solid phase extraction methods require a greater time investment but have decreased matrix effects, which help to increase LC column and mass spectrometer longevity and cleanliness.

POSTER

P38: Low Cost Entry for Automation in Laboratory Settings

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Background/Introduction: In the forensic world many cases are decided based on the laboratory test results. Yet, while forensic testing is a critically important for legal demands, many labs remain constrained by tight budgets and limited resources. This often makes purchasing, replacing, or even expanding a laboratory automation system economically impractical. But continuing pressures including a shrinking number of qualified personnel and increasing test demand driven by introducing new drugs on the street, have only driven the necessity for increased consideration and use of laboratory automation to save on solvents, samples, time and money.

Objectives: To demonstrate the quality and quantity when implementing an entry level automation solution such as the Extrahera™. Evaluating manual Vs. automated extraction; timing, process efficiency, assay variability, and compliance will provide a clear picture of the value add in sample preparation workflow.

Methods: Biotage® Extrahera™ is an automated Sample Preparation Platform equipped with an 8 channel pipetting head, with a built in positive pressure processing functionality. The system is interconvertible between 4 and 8 channel pipetting into 24 (6 x 4 arrangement) columns or 96-well plates, respectively.

DOA calibration standards were prepared by spiking blank whole blood with the analytes of interest. Commercial QCs were used. Eighty blood sample (60 Positive and 20 negative, all results were previously known) were analyzed. The samples were crashed with acetonitrile. After centrifugation, the resulting supernatant was extracted manually and by automation. Times were recorded for each step done by each technique. Cross contamination was examined by verifying negative results.

500 microliters of the supernatant were loaded onto the SPE plate, washed with 1 mL of 4% H3PO4, followed by 1 mL of 50% methanol, and then dried for 1 minute. Compounds were eluted into standard 2 mL collection plates with 2 x 0.75 mL of 78:20:2 (CH2Cl2:IPA:NH4OH).

The samples were then evaporated to complete dryness using SPE-Dry and ACT plate adaptor (to prevent cross contamination from evaporation) under nitrogen at 40°C. They were reconstituted in 100 µL of 95:5 mobile phase A/mobile phase B for LC-MS/MS analysis.

Results: When comparing the timed performance between manual and automated processes, including sample pretreatment and dry down steps, the manual process took 1 hour and 40 minutes while the automated process took 1 hour and 10 minutes. Cross contamination examination showed no contamination occurred through either process.

Conclusion/Discussion: Dealing with blood samples is difficult when done manually. On the whole, the time needed for sample preparation was reduced by more than 25% with the automated compared to the manual process. In case of extracting 3-4 batches per day total time savings can be hours.

The Extrahera™ provided a step by step report that described details for the whole extraction protocol.

The procedure reduced human error, assay variability and eventually laboratory costs. Limited manual intervention with chemicals and specimens also improved safety. Programed methods are saved, and the extraction replicated exactly the same way every time.

POSTER

P39: Extraction and Analysis Methods for Fentanyl and Fentanyl Analogues in Whole Blood and Urine

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Background/Introduction: Fentanyl and fentanyl-related analogues have been identified as the root cause of several notable drug overdoses in recent years. Demand for testing of these drugs has rapidly increased due to the opioid epidemic affecting many cities across both the United States and Canada. Common matrices for testing include urine and whole blood; both of which contain many endogenous interferences. Obtaining optimal analytical results often requires sample preparation to remove these interferences and isolate compounds of interest. Extraction protocols may employ minimal effort, such as with dual-mode extraction (DME+) or supported liquid extraction (SLE+), but may also require more complex methods involving solid phase extraction (SPE) with mixed-mode cation exchange sorbents.

Objectives: Three and four extraction techniques were optimized for commonly tested fentanyl/fentanyl analogues in urine and whole blood samples by LC/MS, respectively. Comparisons were made for recovery, matrix effects, and overall robustness of each protocol.

Methods: Human whole blood and urine samples were spiked with a panel of fentanyl analogues, which were then extracted via dual-mode extraction (ISOLUTE DME+, Biotage), supported liquid extraction (ISOLUTE SLE+, Biotage), silica-based mixed-mode strong cation exchange (ISOLUTE HCX, Biotage), and a polymer mixed-mode strong cation exchange (EVOLUTE EXPRESS CX, Biotage). Each extraction protocol was performed in accordance with the manufacturer's recommendations. Chromatographic separation was achieved on a Shimadzu Nexera X2 UPLC coupled to a SCIEX 5500 tandem mass spectrometer for analysis. Recoveries, process efficiencies and matrix effects were determined by comparing the area counts of extracted samples (pre-spiked), post-extraction samples (post-spiked), and unextracted samples (neat, no matrix).

100 µL of urine or whole blood was used for each extraction evaluation. Each sample was diluted with acidic or basic water (0.1% formic acid in water or 1% ammonium hydroxide) before extraction. The Dual Mode Extraction (DME) procedure utilized acetonitrile for the extraction of the target analytes. The Supported Liquid Extraction (SLE) protocol examined three distinct elution solvents in dichloromethane, ethyl acetate, and MTBE. Both the ISOLUTE HCX and EVOLUTE EXPRESS CX extractions evaluated the use of two elution solvent mixtures; 78:20:2 dichloromethane/isopropanol/ammonium hydroxide or 78:20:2 acetonitrile/ethyl acetate/ammonium hydroxide.

Following extraction, the samples were evaporated using a SPE DRY 96 and reconstituted in 50 µL of a 50:50 mixture of 0.1% formic acid in water/0.1% formic acid in methanol. Samples were then analyzed via LC-MS/MS.

Results: The process efficiencies and matrix effects for each protocol were found to be similar in both urine and whole blood samples. Recoveries using the EVOLUTE EXPRESS CX and ISOLUTE HCX were the highest of the extraction techniques when using the DCM/IPA/NH$_4$OH elution solvent. However, significant signal suppression was noted in the EVOLUTE EXPRESS CX protocol, specifically with whole blood. ISOLUTE SLE+ provided samples with the least matrix effects, although recovery was lower compared to the SPE methods. The pass through extraction of the ISOLUTE DME+ resulted in the least amount of cleanup while still providing adequate recovery. Each method proved to have a reliable LOQ down 0.1 ng/mL

Conclusion/Discussion: Each extraction technique displayed their own merits. It is essential to consider the analytes of interest, limits of quantitation, desired extract cleanliness, compound recoveries, matrix effects, and extraction time when determining the optimal method to implement. For ISOLUTE SLE+, depending on the matrix, either MTBE (blood) or DCM (urine) will provide the cleanest extracts. For ISOLUTE HCX and EVOLUTE EXPRESS CX extractions, the DCM/IPA/NH$_4$OH yielded high recovery, but elevated matrix effects. The ISOLUTE DME+ protocol offers a simple workflow, but will yield slightly dirtier samples.

POSTER

P40: Strategies for Extraction of Drugs from Whole Blood

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Background/Introduction: Whole blood is often the specimen of choice for detection of drugs in the forensic laboratory. Samples are typically extracted to remove interferences and isolate the analytes of interest prior to GC-MS or LC-MS/MS analysis. Options include minimal cleanup using protein removal/phospholipid depletion, dual mode extraction (DME), or a higher level of cleanup using supported liquid extraction (SLE).

Objectives: The objective of this study was to compare recovery of a large drug panel (100 compounds, over ten different drug classes) from whole blood using three extraction techniques.

Methods: Fortified whole blood samples were extracted using protein removal/phospholipid depletion (ISOLUTE PLD+, Biotage), dual mode extraction (ISOLUTE DME+, Biotage) and supported liquid extraction (ISOLUTE SLE+, Biotage) following the manufacturer's protocols. Fortified samples at 50 or 100 ng/mL were extracted and analyzed in triplicate. Samples were analyzed using a Shimadzu Nexera X2 UPLC coupled with a Sciex 5500 tandem mass spectrometer, and process efficiency was determined (1) by comparing area counts of the extracted samples to area counts of a no matrix (neat, unextracted) standard at the same concentration. LC-MS/MS analysis was done in both positive and negative electrospray ionization modes in scheduled MRM mode. Single injections of triplicate sample were analyzed. An 2.7 μm Raptor biphenyl HPLC column (Restek, Bellefonte, PA) was used for chromatographic separation with gradient of 0.1% formic acid in water and 0.1% formic acid in methanol.

PLD samples were extracted with acetonitrile or acetonitrile with 1% formic acid using a "solvent first" approach (crash solvent added to well, followed by addition of whole blood sample). DME was performed by adding the blood sample first, followed by addition of acetonitrile, or 10 μL of formic acid followed by the addition of acetonitrile. Four elution solvents were evaluated for the SLE extractions: MTBE, dichloromethane, 90:10 dichloromethane:2-propanol, and ethyl acetate. Extracted samples were evaporated and reconstituted in 100 μL 90:10 mobile phase A:mobile phase B prior to analysis. 2.5 μL sample was injected.

Results: Protein removal/phospholipid depletion and dual mode extraction yielded good results for most compounds using a simple "pass through" SPE with acetonitrile. Supported liquid extraction also provided good results for most compounds. Recoveries varied depending upon the SLE elution solvent. Some compounds, like gabapentin, pregabalin, and ritalinic acid had lower recoveries but could still be detected.

Addition of acidified acetonitrile did not provide clean samples using PLD, but samples acidified with formic acid using DME provided clean extracts. Ethyl acetate or addition of 2-propanol was needed for recovery of the more polar hydrophilic compounds like amphetamine and benzoylecgonine by SLE.

Conclusion/Discussion: LC/MS or GC/MS analysis of a large drug panel in whole blood is feasible using any of the three techniques, PLD, DME or SLE. Selection of elution solvent must be carefully considered for supported liquid extraction depending on the compounds of interest.

Matuszewski, BK, Constanzer, ML, Chavez-Eng, CM, *Anal Chem,* 75(13) 3019-20 (2003)

POSTER

P41: Laboratory Quality Metrics in a Pain Medication Monitoring Laboratory

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Background/Introduction: While most labs understand the need/requirement for an in-depth quality program, finding reports of parameters to monitor and the resulting data can be challenging. Thus, this presentation focuses on a few aspects of the quality assurance program followed by a successful pain medication monitoring laboratory where both enzyme immunoassay (EIA) and liquid chromatography / tandem mass spectrometry (LC/MSMS) were employed to assess qualitative and quantitative drug testing. Both the kinds of errors and the numbers of errors are examined. The impact of automation on errors is also discussed. Typical data is presented with examples of failures as well as an explanation of how the data was interpreted in pursuit of a "quality result in a timely fashion".

Objectives: The objective of this presentation is to discuss the quality measures used and the data within each measure in a clinical testing laboratory.

Methods: Quality assurance (QA) data were obtained from the same production instruments that were used to test patient samples. The validation of several of the methods discussed herein has been published. The details of these methods are inconsequential to this report, however, the validation process used in all these methods is discussed in great detail by Enders and McIntire (1) and is consistent with both college of American pathologists (CAP) and CLIA recommendations.

Data analysis was completed using Microsoft excel. Performance Testing (PT) results were obtained using the normal production instrumentation and processes. All aspects of the testing process were validated including solid phase extractions (SPE), automated aliquoting (e.g, Tecan and Hamilton robots), and automated data review. New lots of controls and calibrators were prepared and Validated against existing lots of controls and calibrators in production use before release to the production floor

Results: This work focused on 10 categories for corrected reports that should be monitored to maintain quality throughout the laboratory. These include carryover, clerical/review error, contamination/interferent, sample switch – accessioning, sample switch – process, chemistry issue, instrumentation issue, near cut-off, software bug, and pre-analytical. Most errors are determined within the laboratory as opposed to by the client. The number of errors (e.g., corrected reports) by method including the number of tests / method / day and the number of analytes/method is also discussed. A direct correlation between increased automation and decreased error rate is also seen. Another key metric for most laboratories is turn around time (TAT). The average TAT for this laboratory over a year period was 2 days or less

Conclusion/Discussion: A successful quality program must monitor any number of parameters as listed above. Perfection is a state all laboratories should be striving to achieve. However, in the absence of that, the parameters and values presented herein provide measurements for comparison and assessing the adequacy of a quality program.

1. J. R. Enders and G.L.McIntire, A Dilute-and-Shoot LC-MS Method for Quantitating Opioids in Oral Fluid, Journal of Analytical Toxicology 2015 39 (8): 662-667

POSTER

P42: Concordance of Cannabinoid and Opioid Screen and Confirmation Results for Urine Workplace Drug Tests in 2018

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Background/Introduction: With evolving legislation around medicinal and recreational marijuana and THC contaminated CBD products, THC continues to be the most widely detected drug in the American workforce and 2018 was the first year that federal drug tests included the prescription opioid drugs hydrocodone, hydromorphone, oxycodone, and oxymorphone. The prevalence of cannabinoid and opioid positive results, including comparison of screen positive and confirmed positive urine workplace drug testing specimens submitted from January 1, 2018 to December 31, 2018 were analyzed.

Objectives: The aim of this study was to evaluate the prevalence of THCA and opioid confirmed positives in workplace drug testing, and to establish the percent agreement for which presumptive positive specimens reported positive after confirmation, using standard cutoffs for the federally-mandated safety sensitive and US general workforces.

Methods: Initial testing was performed on Beckman Coulter automated chemistry analyzers (5440/5840) using Thermo Scientific DRI and Siemens EMIT II enzyme immunoassays for cannabinoids, opiates, hydrocodones and oxycodones. Confirmation testing was conducted using previously validated methods on Agilent GC-MS or Sciex LC-MS/MS platforms. Data represents workplace drug testing specimens processed in Tucker, GA; Lenexa, KS; and Norristown, PA Quest Diagnostics laboratories.

Results: In 2018, Quest Diagnostics performed 6.2 million US general and 2.3 million federally-mandated tests for THCA. Of those, agreement between screen and confirmation tests ranged from a low of 81.9% (20 ng/mL screen; 15 ng/mL confirm cutoff) to a high of 99.5% (100 ng/mL screen; 15 ng/mL confirm cutoff). Analysis of opioid positivity indicated agreement between screen and confirmation results from a low of 31% for federally-mandated opiate testing (2000 ng/mL screen and confirm cutoff) to a high of 93.4% for hydrocodone-class positivity in both testing populations. Data are represented in Tables 1 and 2 below.

Table 1. Positivity Prevalence of THCA by Cutoff in Federally-Mandated and US General Workforce Testing Populations

Cannabinoids (THCA)- US General Workforce					Cannabinoids (THCA)- Federally-Mandated				
n =	Screen Cutoff (ng/mL)	Confirm Cutoff (ng/mL)	Overall Positivity	Screen/Confirm Agreement	n =	Screen Cutoff (ng/mL)	Confirm Cutoff (ng/mL)	Drug Positivity	Screen/Confirm Agreement
160K	20	10	2.002%	91.6%					
120K	20	15	2.833%	81.9%					
10K	50	10	2.483%	99.3%					
5.9MM	50	15	2.880%	97.7%	2.3MM	50	15	0.893%	97.6%
56K	100	15	2.667%	99.5%					

Table 2. Positivity Prevalence of Opioids by Cutoff in Federally-Mandated and US General Workforce Testing Populations

	Opiates/Opioids - US General Workforce				
n =	Drug	Screen Cutoff (ng/mL)	Confirm Cutoff (ng/mL)	Drug Positivity	Screen/Confirm Agreement
4.4MM	Opiates	2000	2000	0.216%	37.4%
	Codeine			0.132%	
	Morphine			0.122%	
478K	Hydrocodones	300	100	0.551%	93.4%
	Hydrocodone			0.499%	

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n =	Drug	Screen Cutoff (ng/mL)	Confirm Cutoff (ng/mL)	Overall Positivity	Screen/Confirm Agreement
	Hydromorphone			0.456%	
1.8MM	Oxycodones	100	100	0.437%	87.1%
	Oxycodone			0.346%	
	Oxymorphone			0.403%	
Opiates/Opioid- Federally-Mandated					
n =	Drug	Screen Cutoff (ng/mL)	Confirm Cutoff (ng/mL)	Overall Positivity	Screen/Confirm Agreement
2.2MM	Opiates	2000	2000	0.135%	31.0%
	Codeine			0.092%	
	Morphine			0.067%	
2.2MM	Hydrocodones	300	100	0.456%	93.4%
	Hydrocodone			0.418%	
	Hydromorphone			0.366%	
2.2MM	Oxycodones	100	100	0.347%	88.9%
	Oxycodone			0.268%	
	Oxymorphone			0.325%	

Conclusion/Discussion: Overall, specimens tested for cannabinoids, hydrocodones and oxycodones demonstrated good agreement between initial and confirmatory results.   The largest discordance was observed for specimens' presumptive positive for opiates which included morphine, codeine and 6-acetylmorphine analytes in the confirmation panel. Cross-reactivity of prescription opiates likely contributed to the lower percentage of confirmed opiates in the federally-mandated testing group. Additional studies will include a longitudinal analysis of positive prevalence data for cannabinoids and opioids.

Keywords: cannabinoid, opiate, opioid

P43: Free Ammonia as a Predictor of Complications of Acute Alcohol Intoxication

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Introduction: One of the main causes of alcoholic encephalopathy is a violation of the detoxifying function of the liver on the background of prolonged abuse of alcohol and increasing the level of free ammonia, while a high concentration of this metabolite is one of the most important factors in the development of alcohol encephalopathy.

Objectives: to study the indicators of the autonomic nervous system and cognitive functions in patients with acute alcohol poisoning, depending on the level of free ammonia and the duration of alcohol intoxication.

Methods: This study included 96 patients (14 women and 82 men) with acute alcohol poisoning aged from 30 to 55 years admitted in 2015-2018 to the Department of Toxicology of RRCEM against the background of chronic alcohol use. Blood alcohol averaged 1.8 ± 0.9 g/l (determined on a clinical analyzer according to the Karandayev method). The subjects were divided into 3 groups: Group I comprised 34 patients with a high concentration of free ammonia — more than 60 μmol / l (determined on a biochemical analyzer, the norm is 16-32 μmol / l), while the duration of hard drinking in all these patients was more than 10 days. Group II consisted of 32 patients with an average level of free ammonia - 32-60 μmol / l, against the background of hard drinking from 5 to 10 days. Group III consisted of 30 patients with relatively low levels of free ammonia — less than 32 μmol / l and a length of hard drinking for up to 3 days. The degree of cognitive impairment was studied on the MMSE (Mini mental state examination, the norm is 29-30 points) scale by 10 positions in points, the FAB (Frontal Assessment Battery, the norm is 16-18 points) scale by 6 positions, and Reitan's test in seconds (normally less than 100 seconds). In order to analyze ANS: hemodynamic functions were studied: heart rate, systolic and diastolic blood pressure (MAP and DBP), Kerdo vegetative index (VI) according to the formula VI = (1 - D / P), where D is diastolic BP, P is pulse rate in minutes.

Results: In patients of group I, on admission, there was a marked impairment of intelligence level on the MMSE and FAB scales, the total score was 17.5 ± 1.2 and 9.4 ± 1.4, respectively, and the Reitan test was performed with frequent errors and almost only in 2 patients this test was completed, making 246 and 316 seconds, respectively, which corresponds to severe cognitive lesions. With an average level of free ammonia, in patients of group II, the total score of cognitive functions on the MMSE and FAB scales was 23.6 ± 1.2 and 11.2, ± 1.9, respectively, and the Reitan test was 156.5 ± 22.5 seconds, which also corresponds to moderate cognitive lesions. In patients with a low level of free ammonia according to the screening of the level of intelligence on the MMSE FAB scale was 26.7 ± 1.4 and 13.2, ± 1.6, respectively, and the Reitan test was 132 ± 16.4 seconds, i.e. mild cognitive impairment was noted. On admission to the hospital, hypersympathicotonia prevailed in all patients. This was characterized by an increase in the values of systolic and diastolic pressure, heart rate, Kerdo vegetative index. At the same time, in patients with a high level of free ammonia, hemodynamic parameters exceeded the normal limit values by 1.3, 1.2, 1.6, 2.5 times. In the future, 25 (78.1%) patients with high levels of free ammonia developed alcohol delirium.

Conclusion: 1. The patients with alcohol intoxication is a decreasing in cognitive function and hypersympathicotonia in proportion to the level of free ammonia. 2. Patients with patients with a free ammonia level of more than 60 μmol / l have pronounced disorders of the intellect, which require medical correction.

P44: Innocent Victims of Drug Abuser Parents: Acute Cannabis Resin (Hashish) Intoxication due to Accidental Ingestion in Two Toddlers

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Background/Introduction: The annual prevalence of cannabis consumption in Spain in the general population is about 10%. This alarming consumption explained prenatal exposure to cannabis in 5% of newborns according to some studies. Severe poisoning after marijuana ingestion are not frequent among adults but require close monitoring in children.

Objectives: We describe here two cases of severe accidental cannabis poisoning in toddlers. Both girls (16 and 18 months old) were admitted to the pediatric emergencies of two hospitals with decreased level of consciousness. The first one presented vomiting, mydriasis, tachycardia, hypertonia, ataxia, and clonic movements, and the second one had hyperreactivity, hypoglycemia, tachycardia, and irritability. Management including activated charcoal and supportive measures led to complete regression of symptoms. Questioning the parents led to suspecting accidental ingestion of a piece of cannabis resin (hashish). Preliminary toxicological analyses performed at the hospital using MonlabTest® (a simple, one-step immunochromatographic assay) gave positive results for cannabis in urine. After a complaint, the Judge asked for toxicological analyses of urine and blood/plasma samples collected at the hospitals in our laboratory.

Methods: Urine samples were screened by immunoassays (CEDIA) for opiates, cocaine, cannabinoids, amphetamines, barbiturates, benzodiazepines, and methadone. Toxicological analyses gave positive results only for cannabis compounds in both cases. Confirmation and quantification of cannabinoids and metabolites [Δ9-tetrahydrocannabinol (THC), 11-hidroxy-THC (THC-OH), THC-carboxylic acid (THC-COOH), cannabinol (CBN), and cannabidiol (CBD)] in blood/plasma was accomplished by gas chromatography-tandem mass spectrometry (GC-MSMS) using an Agilent 7890B GC interfaced with an Agilent 7000 MS triple quadrupole, multi reaction monitor (MRM) mode. Confirmation and quantification of THC-COOH in urine was accomplished by gas chromatography-mass spectrometry (GC-MS) using an Agilent gas chromatograph 7890A model, coupled with an Agilent MS/EI detector 5975C, selected-ion monitoring (SIM) mode. All samples (1mL) were submitted to a solid-phase extraction, after adding deuterated internal standards and derivatized with BSTFA/TMCS (99/1,v:v) for GC-MSMS and with PFPA/HFIP for GC-MS analyses (1 μl, splitless mode, both cases). Calibration ranges in blood/plasma (GC-MSMS) were as follows: THC and THC-OH: 0.1-50 ng/mL, CBD and CBN: 0.2-50 ng/mL, and THC-COOH: 0.5-100 ng/mL. Calibration THC-COOH range in urine (GC-MS) was 10-1000 ng/mL. Cannabinoids and metabolites (derivatized) were quantified in blood/plasma using the following transitions: THC: **386.2→303.2** and 371.2→305.2; THC-OH: **371.2;→289.2,** and 371.2→305.2; THC-COOH: **488.2→371.2** and 488.2→297.2; CBN: **367.2→310.2** and 367.2→295.2; CBD: **390.2→301.2** and 390.2→319.2; and the following internal standards (IS): d3-THC: **389.2→306.2** and 374.2→308.2; d3-THC-OH: **374.2→292.2** and 374.2→308.2; and d3-THC-COOH: **491.2→**374.2 and 491.2→300.2. THC-COOH (derivatized) was quantified in urine (GC-MS) using target ions at m/z **640,** 625, 489, and at m/z **643,** 628, 492 for d$_3$-THC-COOH as IS. The inter-assay accuracy ranged from 90% to 105% of target and the inter-run imprecision ranged from 5% to 15% for all analytes. Limits of quantitation are coincident with lower calibrators for each analyte.

Results: _Case 1 (Hospital Niño Jesús, Madrid, Spain):_ Plasma: THC: 182.9 ng/mL, THC-OH: 28.3 ng/mL, THC-COOH: 466.2 ng/mL, CBN: 0.2 ng/mL, and CBD: 0.3 ng/mL. Urine: THC-COOH: 217.8 ng/mL.

Case 2 (Hospital Gregorio Marañón, Madrid, Spain): Blood: THC: 51.1 ng/mL, THC-OH: 7.9 ng/mL, THC-COOH: 175.7 ng/mL, CBN: 1.0 ng/mL, and CBD: 0.3 ng/mL. Urine: THC-COOH: 375.0 ng/mL.

Conclusion/Discussion: THC and THC-COOH concentrations found in these toddlers were the highest found in our experience. According to the information provided by the family the two toddlers ingested "hashish" (cannabis resin). Most of these "hashish" seized material samples analyzed in our laboratory (Drug Seized Materials Department) have a THC richness between 30-40%. Infants and toddlers had more severe poisoning presentations than adults indicating the legal implication in child protection by the authorities, courts, etc.

Keywords: Cannabis resin (hashish), acute intoxication, toddlers.

POSTER

P45: Simultaneous Analysis of Ethanol Metabolites (EtG/EtS) and a Marijuana Metabolite in Urine using Modified HILIC/MSMS

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Background/Introduction: Ethanol metabolites, ethylglucuronide (EtG) and ethylsuflate (EtS), are tested using liquid chromatography/mass spectrometry mass spectrometry (LC/MSMS) in negative ionization mode. The primary metabolite of marijuana, tetrahydrocannabinolic acid (THC-A) glucuronide, can be analyzed using either negative or positive polarity LC/MSMS after hydrolysis of the glucuronide conjugate. Both assays are frequently ordered in forensic testing as well as pain medication monitoring. It would seem obvious to test both sets of metabolites in the same method, however, reliable hydrolysis of the THC-A glucuronide must be completed without hydrolyzing the analogous EtG conjugate. While reversed phase chromatography is commonly used for all 3 analytes, development of a hydrophobic Interaction liquid chromatography (HILIC) method proved faster than the corresponding reversed phase method. That method was used in this work as detailed below.

Objectives: The goal of this work was to develop an HILIC/MSMS analytical method for analysis of EtG/EtS/THC-A in a single efficient test. Enzyme hydrolysis was studied to determine reproducible hydrolysis of the THC-A glucuronide without hydrolysis of the ethanol metabolite, EtG.

Methods: Standards and internal standards were purchased from Cerilliant (Red Rock, TX). All other chemicals were received from VWR (Minneapolis, MN). IMCSzyme™ (IMCS, Columbia, South Carolina) was used for hydrolysis. HILIC/MSMS was carried out using a Shimadzu chromatography system coupled to a Sciex 5000 MSMS unit. The UPLC column (50mm x 4.1 mm id) was from Agilent (San Jose, CA). Mobile phase A was 0.1% acetic acid in 20mM ammonium formate in water and mobile phase B was 0.1% formic acid in acetonitrile. An acceptable gradient was determined that provided baseline separation of EtG, EtS, and THC-A from each other and from all interferents.

Results: Chemical hydrolysis of ester linked THC-A glucuronide can be accomplished with base, acid, or a beta-glucuronidase enzyme. While base has been demonstrated to be helpful in negative mode MSMS, hydrolysis in this application was completed using a recombinant beta-glucuronidase from IMCS. Under the mildly acidic conditions used herein, THC-A glucuronide is completely hydrolyzed while the corresponding EtG remains stable for at least 4 days.

A classical isocratic elution under HILIC conditions was not successful as THC-A peak shapes were unacceptable. An acceptable gradient was determined that provided baseline separation of THC-A, EtG, and EtS from each other and from all interferents. Studies demonstrated that a gradient from 90% B to 20% B over 1 minute was adequate to separate the analytes in question on the Agilent column detailed above. A short segment at 1% B was completed to flush the column before reconditioning the column with the starting conditions.

Conclusion/Discussion: Analysis of common metabolites of illicit substances in a single HILIC/MSMS run affords efficient sample preparation and instrument use in the laboratory. In this work, analysis of ethanol metabolites and a marijuana metabolite in a single method reduces both sample preparation time and HILIC/MSMS run time. This is possible because the hydrolytic method used herein is selective under the conditions used such that EtG is not hydrolysed while the THC-A glucuronide is completely cleaved. Further, the extreme hydrophobicity of THC-A results in good separation and reverse elution order using the HILIC conditions herein. Finally, using negative mode MSMS results in lower signal to noise and reduced interferences.

POSTER

P46: Use of an Enzymatic Method in a New Management of Poisoning GHB/GBL in a Hospital Laboratory in France

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Background/Introduction: GHB or gamma-hydroxybutyrate was first synthetized for medical purposes as an anesthesic or for treatment of narcolepsy and has been misused for recreational use like in sexual drug-facilitated crimes in 1990s. In many European countries, GHB is a controlled substance but more recently, new patterns of consumption have been described in festive usage with legal GBL. GBL, colorless industrial solvent, parent compound of GHB, can be carried easily as « doses » by users during parties and has been used in private situations for *chemsex* by seeking endurance and sexual performance. Since the end of 2017 in Paris (France), a recent increase of interest for GHB/GBL has been observed.

Objectives: To respond to this renewed interest, after media coverage of some cases with coma in festive places in Paris at the beginning of 2018, the hospital laboratory of toxicology has changed GHB detection and quantification strategy for rapid response 24/24 to management conditions for intoxicated patients.

Methods: Detection and determination of GHB concentrations in urine and plasma were analyzed in GC/MS method so far. In 2018, an enzymatic assay based on a recombinant GHB deshydrogenase (Bühlmann Laboratories AG) for determination in plasma and urine was adapted on c4000®Architect (Abbott) for routine use 24/24. For each determination, ethanol was determined in plasma or urine to estimate ethanol interference.

Results: The analytical sensitivity of enzymatic assay was <1.5 mg/L whereas the functional sensitivity was 5 mg/L for urine and plasma. Accuracy and precision were estimated on three internal quality controls (n=10) with CV<13.3%. Linearity was observed between 5 and 250 mg/L. Dilution tests 1/10 and 1/50 were validated with patient samples. A comparative study with GHB concentrations in plasma and urine measured with enzymatic assay and GC/MS (n =46) described a very good correlation (r^2> 0,983) for concentrations range between 5 et 10059 mg/L.

From October 2017 to mars 2019, 62 cases of GHB/GBL poisoning have been observed. The majority are men (91.9%) with a median age of 31 years, drug users in festive situations (>45%) and multiconsumers (more than 38% cocaine, 38% benzodiazepines, 26% MDMA and amphetamines and nearly 30% alcoholic drink). Only one death was observed without toxicological circumstances. In population described, mean concentrations of GHB in plasma and urine in the first samples were respectively 214 (+/- 154) mg/L and 1554 (+/- 2130) mg/L.

Conclusion/Discussion: This rapid enzymatic assay on clinical chemistry analyzer after about 18 months seems to be adapted to a 24/24 screening for GHB/GBL intoxications. In 2019, it allows rapid management for intoxicated patients with these new patterns of consumption of GHB/GBL in festive usage, with elevated concentrations.

POSTER

P47: Data-Reduction Middleware for Compound Identification and Quality Monitoring in LC/MS Screening of Psychoactive Substances

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Background/Introduction: Clinical urine drug screening using automated sample preparation coupled with LC/MS can test for the presence of >100 psychoactive substances in hundreds of samples daily but the full benefits can be realized only with an effective data-reduction algorithm for peak identification and careful monitoring for potential errors in both pre-analytical and analytical stages. We describe a lab-developed LIMS-interfaced middleware program which allows one lab technologist to review data from a semi-quantitative 151-compound / 327-MRM LC-QqQ MS acquisition method and, in about 1.5 hrs, report 90 samples of which about 94% are positive for at least one compound.

Objectives: To show the effectiveness of middleware in reducing the need for manual review in clinical LC/MS urine drug screening and in enhancing quality assurance.

Methods: The Microsoft Excel® VBA middleware uses multiple retention times, ion ratios and the presence of expected metabolites/precursors to reliably identify compounds in a graded process with exception-based reporting, though manual review remains necessary for a sub-set of 17 drugs.

The middleware also flags potential errors due to abnormal internal standard signals (n=14), carryover during LC/MS analysis, contamination from adjacent wells during sample preparation (cross-talk: based on marker compounds added in a checkerboard pattern to sample plate), incomplete sample hydrolysis (based on cleavage of added Morphine-Glc-d3), dilute or adulterated samples (based on metabolic ratios of common drugs) and insufficient volume pipetted by automated liquid handlers (based on levels of endogenous hydroxyindoleacetic acid).

Results: We present the middleware's successful application in comprehensive drug screening of up to 630 urine samples daily since May 2016. Initial validation of the middleware showed acceptable correlation with manual review of chromatograms as well as the reference LC/MS method used at the time. Ongoing validation using external quality assurance samples has shown 100% accuracy in identification. To date, the middleware's quality control features have detected cross-talk in five batches and insufficient pipetting in at least one sample, all of which would have been otherwise missed. On average, 2.2% of all samples each month are flagged for suspected carryover and re-tested, with 5% of all re-tested samples (0.11% total) having confirmed/true carryover.

Conclusion/Discussion: Middleware is a reliable and efficient tool for identifying compounds in clinical urine samples, as well as flagging potential analytical and pre-analytical errors.

POSTER

P48: Validation of Cannabinoids in Breast milk using QuEChERS and Ultra-Performance Liquid Chromatography and Tandem Mass Spectrometry (UPLC-MSMS)

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Background/Introduction: Use of marijuana has increased in recent years as a result of legalization, both medically and recreationally. Cannabinoids consumed by a lactating woman may potentially be passed on to their infant through breast milk. Some cannabinoids, particularly Δ9-tetrahydrocannabinol (THC), cannabinol (CBN), and cannabidiol (CBD), are highly lipophilic and move freely into the high lipid content of breast milk. Little information is available concerning the overall developmental effects of cannabinoid exposure through breast milk to infants Presented is a method for the analysis of THC, CBN and CBD in breastmilk.

Objectives: To develop and validate a method for the analysis of cannabinoids in breast milk.

Methods: THC, CBN, CBD, and their isotopically labeled standards were extracted using a modified Phenomenex® roQ™ QuEChERS method and analyzed using ultra-performance liquid chromatography and tandem mass spectrometry (UPLC-MS/MS). As a result of the high lipid content of breast milk, saponification was necessary to improve overall extraction efficiency. Saponification via acids versus alkali was assessed using 1N solutions of hydrochloric acid, acetic acid, nitric acid, sulphuric acid and 1M potassium hydroxide. The modified Phenomenex® roQ™ QuEChERS method was validated. Linearity was assessed from 1 to 100 ng/mL for THC, CBN and CBD. Validation controls were prepared at 1, 3, 20, 80, and 300 ng/mL (1:5 dilution control). Due to the limited access of genuine breast milk for routinely preparing matrix matched calibration and control materials, Enfamil® Premium™ newborn infant formula (0-3 months) was evaluated as a breast milk substitute. Storage stability of cannabinoids in breast milk and formula was determined at 3 ng/mL and 80 ng/mL QC materials for 4, 6, 7, 8 and 12 weeks at refrigerator temperature (5 °C) and freezer temperature (-15 °C). Nineteen authentic samples were acquired and also analyzed. Briefly, samples were prepared as follows: to a tube containing 0.325 g of QuEChERS salt, 750 mcL of sample and 750 mcL of 1N HCl were added. Samples were allowed to sit for 1 minute, then 750 mcL of LC-MS grade acetonitrile (ACN) was added. Samples were shaken in a Biotage® BeadRuptor® 24 for 30 seconds at 5.3 cm/s, then centrifuged at 10,000 x g for 10 minutes. Five hundred microliters of the upper ACN layer was transferred to a roQTM dSPE tube, then vortexed for 3 minutes and centrifuged at 8000 x g for 7 minutes. The upper layer was transferred to a glass insert 96 well plate and analyzed via UPLC-MS/MS with an injection volume of 5 μL.

Results: Saponification of lipids in breast milk with 1N hydrochloric acid improved the overall efficiency and recovery of the method; most notable for THC at 3 ng/mL which increased from 29 to 55 % and 52 to 95 % respectively. All calibration curves for THC, CBN and CBD had $r^2 > 0.9960$. The bias ±20% with CVs <15% were determined for all controls. Matrix suitability of the infant formula was determined by comparing the slopes of infant formula prepared calibration curves with those of genuine breast milk prepared calibration curves; no significant differences were observed for THC, CBN and CBD. Cannabinoids in breast milk and formula were stable at 5 °C and -15 °C for up to six weeks, and when stored -70 °C, they were stable up to 8 weeks. Two of the genuine specimens were determined to be positive for THC.

Conclusion/Discussion: The modified Phenomenex® roQ™ QuEChERS extraction method and UPLC-MS/MS analysis were robust and reliable for the quantitation of THC, CBN and CBD in breast milk. Enfamil Premium new born formula is an acceptable alternate matrix.

POSTER

P49: Drugs of Abuse Screening and Quantification Using Paperspray Technology

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Background/Introduction: Paper spray is a rapid analysis technique which is particularly beneficial for the analysis of compounds in biological matrices, such as blood and urine. The technique offers a strong advantage over traditional methods which rely on chromatographic separation with their associate solvent use and time required for sample preparation and analysis.

Objectives: Demonstrate that paper spray technology can be used as a rapid way to screen and quantitate drugs of abuse in blood and urine in a robust and reproducible manner.

Methods: For the screening method, a total of 19 compounds (opiates, amphetamines, cocaine and PCP) were spiked into donor urine along with internal standards. Three concentration levels (n=5) were prepared at five times below the LOQ, at the LOQ, and three times above the LOQ for the screening of cocaine and its metabolite benzoylecgonine. These standards were spiked into urine and covered a concentration range of 5-1000 ng/mL. QC samples were prepared at three additional concentrations. Eight microliters of each respective urine sample was spotted onto a paper spray cartridge for analysis. Samples were resuspended on the paper using rewet and spray solvents before being introduced into the mass spectrometer. Data was acquired for 1 minute per sample, and each concentration level was measured 5 times for screening, and 3 times for quantitation.

For the EDDP analysis in whole blood, EDDP was spiked into whole human blood at various concentrations to yield a nine point curve ranging from 1.75 to 500 ng/mL and three quality controls at concentrations of 50, 100, and 200 ng/mL. A total of 240 samples, consisting of either calibrators or quality controls, were spotted onto the paper cartridges at 8 µL each and were oven dried for 30 minutes at a temperature of 50 °C. Sample plates were then loaded onto the Thermo Scientific™ VeriSpray plate loader, which holds up to 10 paper spray plates (24 paper strips per plate), and connected to a VeriSpray Ion source and a Thermo Scientific™ TSQ Quantis mass spectrometer where it was then analyzed and assessed for robustness.

Results: All 19 compounds met their respective screening cutoff levels (≤15% RSD of response ratio at the cutoff level, and the absolute AUC at least 4x higher than the AUC of the matrix blank). For Cocaine and Benzoylecgonine, curves were constructed by integrating the resulting chronogram from 0.1 – 0.9 min. Both curves were linear over the tested concentration range and met precision (< 15%) and accuracy (+/- 20%) requirements at the lowest calibrator level of 5 ng/mL (LOQ). All QC samples (low, middle, and high) were within 10% accuracy and 5% RSD for both, cocaine and benzoylecgonine. The lower limit of quantification (LLOQ) for the TSQ Quantis for EDDP was 3.5 ng/mL and was set to the lowest calibration standard analyzed that yielded < 20 % accuracy and < 15 % CV for 9 replicate injections. The labeled d3-EDDP present in all 240 samples was monitored for the entire run and had a peak area RSD of 32% spanning the entire run and a RSD of 1.5 % when comparing the peak area ratio of the labeled and unlabeled EDDP standard at 50 ppb level. Additional results will be collected on the TSQ Altis and TSQ Fortis.

Conclusion/Discussion: Paper spray technology can be used as a rapid screening method for drugs of abuse directly from dried urine spots. No sample preparation other than spiking an internal standard and spotting the urine samples on the paper is required and carryover is not an issue. Methods are less than one minute and the technology has shown to be robust with quantitative results obtained for EDDP in whole human blood for 240 samples run continuously without cleaning.

POSTER

P50: Targeted Forensic Screening and Semi-Quantitation of Drugs in Plasma using High-Resolution Accurate-Mass Detection and On-Line Sample Preparation

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Background/Introduction: In forensic toxicology, it is desirable to screen for as many compounds as possible as efficiently as possible. Traditional immunoassay-based methods require several tests to cover even a small range of drug classes. Gas chromatograph-based methods often require derivatization steps for adequate sensitivity and are limited in the range of compounds they can analyze. Liquid chromatography coupled to mass spectrometry (LC-MS) offers the ability to screen for a wide chemical range of compounds with a single analysis.

Objectives: The objective of this work was to develop an Orbitrap mass spectrometer-based LC-MS method that could screen for ~1500 compounds of forensic interest in a single chromatographic run. The screening method uses a high-resolution spectral library and compound database that includes the exact mass, chemical formula, retention time, and exact masses of main fragments for each compound. The method can both screen for all compounds as well as provide verification with semi-quantitation. A partial method verification was performed using 41 representative drugs in plasma. The 41 compounds can be used as a basis for the method verification since they cover different drug classes, retention times and polarities.

Methods: Two different variations of the analytical method were tested, one used only HPLC, with a run time of 15.5 minutes, and the other used on-line extraction prior to HPLC separation, with a run time of 16.75 minutes. To generate the spectral library and compound database, ~1500 standard solutions were analyzed with both methods to obtain retention times and MS/MS spectra.

The limits of detection (LOD), the limits of quantification (LOQ), and the limits of identification (LOI) were determined for 41 compounds (including opiates, benzodiazepines, anti-depressants. amphetamines, and anti-psychotics) in spiked plasma utilizing the on-line extraction approach. Pools of the compounds were spiked into 3 different lots of plasma. Concentrations ranged from 0.1 to 250 ng/mL or from 10 to 5000 ng/mL, depending on the pool. Plasma was processed by addition of internal standards and one volume of acetonitrile before injection. Compound were detected on a Q Exactive Focus high-resolution accurate-mass mass spectrometer. An inclusion list was used to trigger MS^2 fragmentation spectra. The LOD was defined as the lowest concentration for which a peak was observed in all 3 plasma matrices tested. The LOQ was defined as the lowest concentration for which quantitation bias was <20%. The LOI was defined as the lowest concentration for which a compound passed: m/z of the parent (< 5 ppm), isotopic pattern match, fragment ion presence, and MS^2 spectra matching.

Results: A compound database and a spectral library for the screening of the ~1500 compounds were developed on the Q Exactive Focus mass spectrometer. A partial analytical method verification was performed in plasma. LODs ranged from 0.5 to 100 ng/mL. LOIs ranged from 5 to 1000 ng/mL.

Conclusion/Discussion: A compound database and a spectral library for the forensic screening of ~1500 compounds were created using online sample clean-up system followed by HPLC separation and high-resolution accurate-mass mass spectrometry. The screening panel includes compounds of forensic toxicological interest over a wide range of chemical classes such as drugs of abuse and their metabolites, antidepressants, beta-blockers, antibiotics, pesticides, novel psychoactive substances, and others. The method analyzed both positively and negatively ionized species in a single analytical run. The versatility of this method enables easy addition of new substances to the screening panel. Analytical validation for the online sample clean-up method was performed on 41 compounds spiked in plasma matrix. The final method provided screening and quantitation in a single analysis.

POSTER

P51: A Simple LC-MS/MS Method for Hair Drugs of Abuse Quantification

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Background/Introduction: Drugs of abuse testing in hair samples is an emerging diagnostic tool for forensic testing, drug compliance testing, and drug treatment monitoring. Compared to urine and blood, hair analysis has some advantages. It could determine recent drug use; Or it could provide a longer detection window for personal drug history; and a view into long-term drug history through segmental analysis. It has other beneficial aspects, such as ease of storage and shipment.

Objectives: We aimed to develop an LC-MS/MS method with easy and quick sample preparation to analyze 16 common illicit drugs in hair, including morphine, O6-Acetylmorphine, amphetamine, methamphetamine, benzylecgonine, CBD, CBN, cocaine, codeine, ephedrine, heroin, ketamine, methadone, mathcathinone, MDMA, tramadol.

Methods: 0.2 g hair was cut and grounded after being washed twice by 5 mL water and 5 mL acetone. 0.1 g±0.001g grounded hair was weighed. The analytes were extracted by sonication in 1 mL mixture of methanol and acetonitrile. After centrifugation, the supernatant was diluted with water and injected into LC-MS/MS.

The HPLC Phenyl Hexyl column was used to separate compounds with a 15.5 min LC gradient. A：0.1% formic acid, 2mM ammonium formate, B：0.1%formic acid, 2mM ammonium formate with 1% water in 50/50 methanol/acetonitrile. 0-1 min, 99% A；1-10 min, 99%-1% A；10-11.5 min, 1% A；11.5-11.6 min, 1%-99% A；11.6-15.5 min, 99% A. Triple quadruple mass spectrometer was used for quantification under SRM mode. Data was analyzed by quantification software.

Results: We tested two different grounding methods and several different extraction solvents. The whole extraction process took less than 30 minutes. The method had baseline chromatographic separation for all compounds and achieved desirable limit of quantification. Our method demonstrated the lower limit of quantification ranged from 2 ng/g to 10 ng/g with good linearity up to 2 mg/g for the monitored compounds. We also evaluated method reproducibility with the RSD% from 1.4-7.6%.

Conclusion/Discussion: We demonstrate an easy and quick LC-MS/MS method to quantify 16 hair drugs of abuse. We evaluated our method using several positive samples. The lowest positive result for methamphetamine was 66 ng/g in hair.

P52: Analysis of Drugs in Serum and Urine using a Small, Innovative Triple Quadrupole LC/MS

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Background/Introduction: Analyzing drugs in urine and serum are common high-throughput analyses for forensic toxicology laboratories, where reducing cost per sample and instrument downtime is key. Presented here is a sensitive and robust method for analyzing over 60 drugs in human serum and urine using a small and innovative triple quadrupole LC/MS (LC/TQ) with electrospray ionization (ESI) in a fast and robust method.

Objectives: The purpose of this method is to offer robust and sensitive analysis for quantifying drugs in human urine and serum using a small and cost-effective configuration of an LC/TQ platform with a standard ESI source.

Methods: Drug compounds, including opiates/opioids, stimulants, benzodiazepines, and other drug classes, were spiked into human urine and serum prior to sample preparation. Urine samples were diluted prior to analysis, and serum samples were prepared using an acetonitrile crash and then also diluted. Analytes were separated using a C-18 column, 2.1 × 100 mm, 2.7 µm. Compounds were analyzed in a quick 7-minute method in dynamic MRM mode on an LC/TQ with an ESI source.

Results: Excellent sensitivity was observed, with all compounds meeting required quantitation limits in their respective matrices. Exceptional precision was also observed, with RSD% <20% for all compounds analyzed at their quantitation limit. The system robustness of the LC/TQ configuration ESI source was also evaluated for 26 drugs in human serum matrix with a six-day continuous run analyzing 1625 individual injections of QC and calibration curve samples. Average RSD% for both raw peak area and calculated concentration of 1400 QC samples were 4.3 and 4.4% respectively for the 26 analytes.

Conclusion/Discussion: This method offers a sensitive, robust and cost-effective way to quantify targeted drugs in human serum and urine, highlighting advancements and innovation in the forensic toxicology analysis space.

POSTER

P53: Quantitative Analysis of Gamma Hydroxybutyrate in Human Serum Using LC-MS/MS

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Background/Introduction: Gamma hydroxybutyrate (GHB) is an endogenous compound and a drug commonly used by bodybuilders. It is also abused by teens and young adults at bars and parties and is often put in alcoholic beverages. GHB is referred to as "club drug" or "date rape" drug. Victims become disabled due to its sedative effect and are unable to resist sexual assault. High doses of GHB, may result in profound sedation, seizures and severe respiratory depression and in extreme cases, death. Patients are commonly admitted to hospital emergency rooms for treatment. GHB and its analogues are commonly encountered in driving under the influence of drugs (DUID) cases and in post mortem toxicology when poisoning deaths are investigated. The quantitative identification of the drug is indispensable in sexual assault cases or forensic medical investigation of drug intoxication.

Objectives: The aim of this communication is to present a comprehensive method for the quantitation of GHB in human serum employing solid phase extraction (SPE) in conjunction with LC-MS/MS

Methods: A polymeric SPE chemistry, Strata-X Pro in 30 mg, 96 well plate format was utilized for sample extraction. A quick, two step sample prep protocol was employed, that doesn't require any conditioning or equilibration of the SPE phase. Two hundred µL serum sample was treated with 600 µL acetonitrile/methanol (90/10). The supernatant obtained from the precipitated sample, via centrifugation, was passed through the SPE device and collected for injection. A Luna 3u, HILIC column in 150x2.0 mm dimension, employed for chromatographic analysis, enabled direct injection of the extracted sample constituting 75% organic. The prescribed protocol circumvents the need for a dry down or reconstitution step and makes it quick and easy to implement in a laboratory workflow. A SCIEX API 4500 instrument under ESI in positive ionization mode was used for MS analysis. Mobile phase consisting of 100 mM ammonium formate and acetonitrile was utilized in a 2-minute gradient run. A Kinetex 5u, C18, 50x2.1 mm, dimension column was also incorporated to showcase successful removal of endogenous phospholipids from extracted samples.

Results: The Strata-X-Pro product is specifically designed to capture the phospholipids, proteins and other matrix interferences, utilizing the 'catch and release' mechanism. About 90% of the phospholipids were removed from extracted sample while recovering 75% of analyte. Calibration curve constructed with spiked serum sample covering the concentration range from 1-300mg/L. The cutoff concentration recommended is 50mg/L, in order to discriminate between the endogenous production and antemortem ingestion of GHB. The two concentrations (low and high) of QC samples analyzed, produced a precision below 20% with an accuracy ranging between 80-120% for 6 replicate samples.

Conclusion/Discussion: The developed extraction technique resulted in high recovery of GHB with maximum removal of the phospholipids, which are the major cause of matrix effect and MS instrument downtime. The prescribed Strata-X-Pro method illustrates a fast workflow and yet a cleaner extraction procedure, while a Luna HILIC column was used in conjunction.

POSTER

P54: Performance Evaluation of Synthetic Urine Kit for the Detection of Synthetic Urine in the Workplace Drug Testing Program

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Background/Introduction: Synthetic urine (SU), which has screened negative on our immunoassay analyzers and passed specimen validity testing, is now commercially sold as a "novelty" and/or "fetish" product to the general public. Due to the challenges of identifying substituted and/or adulterated specimens accessioned in a high throughput drug testing laboratory, we evaluated the performance of a commercially available SU detection kit with our screening analyzers.

Objectives: Determine the feasibility and reliability of an automated synthetic urine detection kit in a high-throughput workplace drug testing.

Methods: Synthetic urine kit from Sciteck was donated to the Forensic Toxicology Drug Testing Laboratory (FTDTL), Tripler Army Medical Center, Oahu, HI. The kit was evaluated in the Beckman AU5820 Screening Analyzer. Per the manufacturer's insert, any normalized value of ≤30 mg/dL is reported as *invalid*. Samples were poured in 16 x 125 mm polypropylene tubes and stored at room temperature. The analyzer was calibrated in the morning prior to running any samples. Performance was evaluated with five batches. Batch 1 included Sciteck normal and calibrator controls, deionized water, five negative authentic urine samples, seven SU (purchased from smoke shops on Oahu, HI), QC samples in Microgenics SU and UTAK authentic urine prepared in house. Batches 2, 3, and 4 each consisted of 116 service member (de-identified) samples and four in-house QC samples (fortified in UTAK or Microgenics). Batch 5 consisted of authentic urine samples from two donors collected from first void of the day (T0) to T+120 hours later, , adulterants (50:50 bleach:water, beer, fish sauce, soapy water, coffee, Sports drink) and six de-identified service member samples flagged for suspected adulteration during accessioning. Batches 1 and 5 were analyzed in three separate runs.

Results: Sciteck (authentic urine) normal controls elicited values of ≥319 mg/dL, while water and QC in Microgenics SU measured at ≤17 mg/dL. QC in UTAK (authentic urine) read from 70 to 210 mg/dL. In comparison, all SU read ≤59 mg/dL. Donor urine samples collected as first void read >1000 mg/dL, while urine samples collected in the middle of the day (after consuming >32 oz of water) read between 25 and 93. Adulterants had readings ranging from -14 (50:50 water:bleach) to 3029 mg/dL (coffee). Suspected adulterated samples gave values between -21 to 150 mg/dL. Values obtained from batches 2, 3, and 4 ranged from -17 to 2443 mg/dL (median = 463 mg/dL, mean =514.8 mg/dL).

Conclusion/Discussion: Synthetic urine products have become popular among those who are subjected to drug testing as products that can "fool" the drug test. With limited synthetic urine detection kits commercially available and compatible with the AU5800 Beckman analyzers, the the Sciteck SU kit was able to distinguish between SU and authentic urine. However, diluted samples (in- or ex-vivo) may show as "invalid" while some adulterated samples can be construed as authentic urine. We believe further evaluation of the kit alongside with an adulterant detection kit can identify true adulterated samples. Automation without subjective laboratory personnel interpretation would be a great benefit for any workplace drug testing laboratory. To deter service members as well as civilians from cheating the drug testing program and improving testing accuracy, synthetic urine detection reagents may need to be added to the drug testing panel.

POSTER

P55: Separation of Opiates and Their Oxime Derivatives Using Differential Ion Mobility Tandem Mass Spectrometry (DMS-MS/MS)

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Background/Introduction: Differential ion mobility spectrometry (DMS) is a technique for post-ionization differentiation that separates ions in the gas-phase based on the ions' mobility in high and low electric fields. DMS-based separation methods combined with mass spectrometry are effective at the rapid separation and detection of ions at atmospheric pressure. Morphine and codeine are naturally occurring opiates found in the *Papaver somniferum*, poppy plant. Semi-synthetic opiates are derived from compounds in the poppy plant and include hydromorphone, hydrocodone, oxycodone, and oxymorphone. Opioids produce a wide variety of centrally- and peripherally- mediated responses including analgesia, sedation, respiratory depression, and euphoria by binding to the mu, delta, and kappa opioid receptors throughout the body. Opioid analgesic compounds are the most commonly used pharmacologic agents for the management and treatment of moderate to severe pain. In addition, the non-medical use of opioids is one of the fastest growing forms of drug abuse in the United States that has ultimately led to the current opioid crisis.

Objectives: To develop a differential ion mobility spectrometry tandem mass spectrometry (DMS-MS/MS) method for the separation of opiates including morphine and its isobar hydromorphone, codeine and its isobar hydrocodone, oxycodone, oxymorphone, and 6-monoacetylmorphine (6-MAM) with no column chromatography.

Methods: Separation was performed in a SelexION® DMS component from Sciex coupled to a QTRAP 6500+ with an IonDrive Turbo V source for TurboIonSpray®. The separation voltage was kept constant at 3900 V while the compensation voltage was ramped from -45 to 15 V over 0.5 min in MRM mode. Deprotonation potential was 100 V for all opiates. The transitions monitored (*m/z*) with collision energies (eV) in parentheses are shown in the table below. 12 chemical modifiers of varying viscosities, dipole moments, and polarities and a mixed modifier of 50:50 isopropanol: toluene were evaluated for resolution of the opiates. The modifiers that produced the largest overall resolution was then used to separate the oxime derivatives of hydrocodone and hydromorphone from codeine and morphine.

Opiate	Transition ion (*m/z*)	Oxime transition (*m/z*)
Morphine	286 > 152 (78)	N/A
Hydromorphone	286 > 185 (40)	301 > 209 (16)
Codeine	300 > 115 (100)	N/A
Hydrocodone	300 > 199 (40)	315 > 255 (37)
6-MAM	328 > 165 (51)	N/A
Oxycodone	316 > 212 (58)	331 > 313 (24)
Oxymorphone	302 > 227 (38)	317 > 299 (23)

N/A, not applicable

Results: Isopropanol resulted in the complete resolution of oxymorphone and oxycodone from the other opiates, and toluene produced the greatest resolution of 6-MAM from other the opiates. The 50:50 mixture of toluene and isopropanol resulted in the overall best resolution, but co-elution of the isobars morphine/hydromorphone and codeine/hydrocodone occurred regardless of modifier. Formation of the oxime derivatives prior to analysis resulted in some separation of the isobars using isopropanol, toluene, and the 50:50 isopropanol: toluene mixture as chemical modifiers.

Conclusion/Discussion: In the presented method, separation of opiates was achieved with no column using DMS-MS/MS. Transport gas modifiers can increase resolution of compounds. 50:50 isopropanol: toluene most effectively resolved all of the opiates, demonstrating mixed modifiers have the potential to provide greater separation than single modifiers. Partial separation of the isobaric opiates was achieved by forming the oxime derivatives prior to analysis.

Keywords: Opiates, differential mobility spectrometry, DMS-MS/MS

P56: Method Validation for the Simultaneous Quantification of 13 Commonly Encountered Stimulants in Whole Blood by LC-MS/MS

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Background/Introduction: In 2018, a total of 1029 cases were submitted by the Rhode Island Center for the Office of State Medical Examiners for analytical testing by the Rhode Island Forensic Toxicology laboratory. These submissions included 230 cases that were positive for stimulants (22.3%) with a majority (154) related to cocaine and/or metabolites (67%). This represented the second most commonly encountered compound class, trailing only opioids. Liquid chromatography/tandem mass spectrometry (LC-MS/MS) was employed to validate a 13-analyte panel for in-house stimulant analysis to increase the number of stimulants quantified in post-mortem specimens and reduce the time to obtain laboratory results. This method also evaluates presence/absence of these same compounds in specimens submitted for impaired driving cases.

Objective: Develop and validate a LC-MS/MS method that allows for a single sample preparation and quantitation of amphetamine, phenylpropanolamine, pseudoephedrine/ephedrine, MDA, methamphetamine, MDMA, phentermine, MDEA, methylphenidate, ritalinic acid, cocaine, cocaethylene, and benzoylecgonine in whole blood.

Methods: 800 µL of 0.1 M zinc sulfate and ammonium acetate solution was added to a microcentrifuge tube followed by the addition of 200 µL whole blood aliquots of samples, controls, or calibrators and vortexed 5-10 seconds. A 100 µL mixture of complementary deuterated internal standard (IS) was added to prepared blood samples then vortexed for 30 seconds. Samples were centrifuged, and the supernatant diluted with 900 µL of 4% phosphoric acid. Pretreated samples were extracted by solid-phase extraction (SPE) and decanted with a 96-well positive pressure manifold. Samples were washed with 500 µL of 2% formic acid with 100 mM ammonium formate and then methanol; dried at high positive pressure for 3 minutes and eluted with methanol:acetonitrile (75:25) containing 5% strong ammonia. Samples were evaporated to dryness and reconstituted with 100 µL of water:acetonitrile:formic acid (97:2:1) for analysis. Analysis was performed by LC-MS/MS using a Waters ® ACQUITY UPLC H-Class LC system coupled to a Waters ® Xevo TQD. Mobile phases were: 0.1% formic acid in Milli-Q water (Solvent A) and 0.1% formic acid in acetonitrile (Solvent B). The optimized flow rate was 0.4 mL/min using gradient elution with initial conditions of 98:2 (Solvent A: Solvent B) increased to 47.2:52.8 over 6 minutes then returned to 98:2 over 0.5 minutes. The system re-equilibrated for 1.5 minutes with an entire cycle time of 8 minutes. Column temperature was maintained at 30°C and the autosampler maintained at 5°C. Injection volume was 5 µL and the needle was purged with methanol:water (50:50) between injections and washed with water: methanol: acetonitrile: isopropanol (1:1:1:1). Two transitions were monitored for each analyte and internal standard. Validation was performed using SWGTOX and the RI Forensic Toxicology laboratory guidelines.

Results: All analytes fit a linear 1/x weighted curve with an $R^2 \geq 0.99$. The LOD ranged from 1-10 ng/mL (analyte dependent). All LOQ's were 10 ng/mL. Percent bias and %CV for all analytes was within acceptable range of +/- 20%. Analytes in samples previously identified quantitatively by other methods demonstrated 100% concordance with this method. Matrix effects and ionization effects were noted and controlled for using matrix matched controls and calibrators with deuterated IS. No significant carryover or interference from drugs of abuse was observed. Extracts were stable 3 days and quantitated at a 1:2 and 1:4 dilutions.

Conclusion/Discussion: A method for the simultaneous identification and quantitation of 13 stimulant compounds in whole blood was successfully validated using SPE and LC-MS/MS.

Keywords: Stimulants, LC-MS/MS, Whole Blood

POSTER

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P57: Validation of a Method for Quantitative Analysis of Multiple Stimulants in Blood using Agilent Captiva© EMR- Lipid cartridges and LCMSMS

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Background/Introduction: Agilent Captiva© Enhanced Matrix Removal (EMR)—Lipid cartridges use a unique sorbent material that selectively removes major lipid classes from sample matrix without unwanted analyte loss. The lipid removal mechanism is a combination of size exclusion and hydrophobic interaction between the sorbent and long aliphatic chain of the lipids. This sample cleanup process reduces ion suppression/enhancement on target analytes, which in turn increases reliability and reproducibility. This method replaces four SPE GC/MS methods (methamphetamine/amphetamine, cocaine/benzoylecgonine/cocaethylene, MDMA/MDA, and phentermine) and adds in-house quantification for ephedrine and pseudoephedrine.

Objectives: The objective of this work was to develop and validate a liquid chromatography tandem mass spectrometry (LCMSMS) method for the quantitation of commonly seen stimulants in postmortem and antemortem blood using Agilent Captiva© EMR-Lipid cartridges, following SWGTOX method validation guidelines.

Methods: A protein precipitation and sample cleanup procedure with an Agilent 1200 Infinity Series High Performance Liquid Chromatography (HPLC) system combined with an Agilent 6430 Triple Quadrupole LCMS system was developed and validated to quantitate methamphetamine, amphetamine, MDMA, MDA, phentermine, cocaine, benzoylecgonine, cocaethylene, ephedrine, and pseudoephedrine. Samples were prepared by using Agilent Captiva© EMR-Lipid cartridges. Samples were prepared by adding deuterated internal standards to 250 μL of sample. The addition of acetonitrile/methanol with 1% ammonium hydroxide to all samples was used to form a protein precipitate. Samples were vortexed, and then centrifuged, followed by transferring the crash solvent to the cartridges. Elution of the crash solvent occurred under low pressure followed by the addition and elution of acetonitrile. 1% hydrochloric acid in methanol was added to the elution before drying down. Samples were reconstituted in mobile phase A (LCMS grade water with 0.1% formic acid and 5mM ammonium acetate) and injected onto the LCMSMS system. HPLC separation occurred using an Agilent Poroshell 120 EC-C18 column (2.7 μm x 2.1 x 100mm) and gradient elution at 40°C. The method validation followed SWGTOX guidelines for accuracy, bias and precision, limits of detection and quantitation, calibration model, selectivity/specificity, carryover, matrix effects/ion suppression, dilution integrity and stability.

Results: Accuracy and bias/precision were determined not to exceed CV values of +/- 20% for all compounds. Between run mean accuracy ranged from 94% - 111%. Between run precision was less than 15% for all compounds (range 4.4% - 12.6%). The limit of detection was determined to be 4.0 ng/mL for all targets except cocaine and cocaethylene which were 2.0 ng/mL. The lower limit of quantitation was determined to be 10 ng/mL for all targets except amphetamine, methamphetamine, and benzoylecgonine which were 20 ng/mL. The upper limit of quantitation was 1000 ng/mL for all targets except amphetamine, methamphetamine, and benzoylecgonine which were 2000 ng/mL, with a combination of linear and quadratic 1/x weighted calibration models. There was no significant interference from matrix effects or common drugs of abuse observed. Recovery was between 33-86% with lowest seen in methamphetamine.

Conclusion/Discussion: Using the Agilent Captiva© EMR-Lipid cartridges and LCMSMS a method for quantitation of stimulants in blood was successfully developed and validated. The cartridges provided a relatively simple and fast cleanup of the samples, which, exceeds current capability and efficiency of our current methods. Future endeavors may include development of an automated extraction process with use of the Captiva© EMR-Lipid 96 well plates and the Hamilton Starlet.

POSTER

P58: Comparison of Glucuronidase Hydrolysis Efficiencies in Opioid Patient Urine Samples

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Background/Introduction: Opioid analgesics are used in the treatment of moderate to severe pain management. Examples of these drugs include morphine, hydromorphone, and codeine. As part of pain management therapy, urine monitoring for pre-scribed opioids is required. Since these drugs are mainly excreted in urine as glucuronides, inclusion of a hydrolysis step is neces-sary to provide adequate detection. One common method for hydrolysis is the use of glucuronidases, either as crude extract or as recombinant enzyme, the latter being more efficient and cleaner. Here we present the results that lead us to the replacement of a crude snail extract currently used in a production method with a recombinant glucuronidase.

Objectives: Determine the best recombinant glucuronidase solution to replace an H. pomatia enzyme extract used in an LC-MS/MS opioid production method.

Methods: Calibrators, QCs, and patient specimens were prepared for analysis in 96-well plate format using Freedom EVO® liquid handling system. Samples (350µL) were diluted with internal standards (25µL) and a glucuronidase master mix solution (320µL). Four enzymes from different vendors were tested at their recommended hydrolysis conditions- Sigma H. pomatia Type H-3 at 65°C for 2 hours; IMCS E1F at 65°C for 1 hour; IMCS RT at room temperature (21°C) for 15 minutes; Kura BGTurbo-gf at 65°C for 15 min-utes. Each master mix was adjusted to give an enzyme:sample ratio of 1:10. Hydrolyzed samples were loaded onto Phenomenex Strata X-Drug B plate preconditioned with TRIZMA buffer (pH 8.1), followed by a two-step wash with 0.1M acetate buffer (pH 4.0) and methanol and extracted with 70:25:5 ethyl acetate/2-propanol/ammonium hydroxide. Extracts were dried down with heat-ed nitrogen and reconstituted with 87:1.99:0.1 water/acetonitrile/formic acid. Samples were then analyzed on a Waters Acquity UPLC/TQD platform using a Phenomenex Kinetex XB-C18 column (2.1x50mm, 1.7um) with 0.1% formic in water and acetonitrile as mobile phases. Morphine-3-glucuronide in BioRad Low C3/C4 quality control solutions (375 and 4000ng/mL post-hydrolysis concentration) were used as quality controls. The resulting patient urine concentrations of codeine, morphine and hydromorphone of each of the recombinant enzyme were compared against those obtained with H. pomatia.

Results: Thirty-six patient samples that previously tested positive for one of three opioids were selected for recombinant enzyme candidate screening. All recombinant enzymes exhibited increased concentrations for morphine and codeine, with most samples falling either within ±20% or >20% of original results. For hydromorphone concentration, Kura BGTurbo-gf and IMCS E1F showed higher variability, with a greater proportion of samples underreporting by <20% from the original results (75% and 58%, respective-ly; n=12), while IMCS RT had a higher proportion fall within ±20% or >20% original results (83%, n=12). IMCS RT was then chosen for an expanded opioid patient screen due to its higher activity; 133 patients in total were subsequently randomly selected and their opioid concentrations were again compared to those obtained in the original method (see table below).

Analyte	n	<20%	±20%	>20%	Detected where current meth-od reported no detect
Morphine	62	4	23	29	6
Hydromorphone	67	6	13	38	10
Codeine	40	1	0	27	12

Conclusion/Discussion: IMCS RT displayed the greatest proportion of results within ±20% or >20% compared to the other enzymes and proved adequate for replacement of the snail extract upon expanded patient testing. When selecting enzymes for optimal hydrolysis, it was noted that real urine patient samples contribute more information compared to reference materials. Randomly screening patient samples was also of benefit as it revealed additional samples which were previously undetected. Higher activity with this new enzyme raised detection rates of patients towards the lower end of quantitation.

POSTER

P59:A Biological Evaluation in Acute Glufosinate Ammonium Poisoning: Determination of Glufosinate Ammonium, 1-Methoxy-2-Pronanol and Sodium Laureth Sulfate in Blood and Cerebrospinal Fluid

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Background/Introduction: Glufosinate ammonium is one of the most frequently detected herbicides in poisoning cases where autopsies performed in the National Forensic Service in Korea. A previous study regarding glufosinate ammonium toxicity showed that each of ingredients can cause neurological complications directly or can influence cell permeability of other ingredients. Glufosinate ammonium herbicides contain glufosinate ammonium, 1-methoxy-2-propanol and sodium laureth sulfate (SLES, formulation according to BASTA SL200® SAFETY DATA SHEET) are on sale worldwide. Proposed mechanisms of glufosinate ammonium acute toxicity include glufosinate ammonium as an active ingredient causeing excessive stimulation of the NMDA (N-methyl-D-aspartate) receptor as glutamate analogue, inhibiting glutamate synthetase and increasing serum ammonia levels. SLES as a surfactant causes gastrointestinal irritation and increases the blood-brain barrier (BBB) permeability of glufosinate ammonium. Distributions of 1-methoxy-2-propanol and SLES in blood and CSF have not been evaluated in cases of glufosinate ammonium poisoning.

Objectives: In this study, an analytical method for the determination of 1-methoxy-2-propanol and SLES in biological fluids including blood and CSF was developed. The levels of glufosinate, 1-methoxy-2-propanol and SLES were evaluated for biological monitoring including distribution in blood and CSF. A liquid chromatography-tandem mass spectrometry method (LC-MS/MS) was used for the analysis of glufosinate and SLES. A gas chromatography mass spectrometry method (GC/MS) was used for the identification and quantitation of 1-methoxy-2-propanol. A developed method was applied to blood and cerebrospinal fluid (CSF) from a 74 year-old woman diagnosed with acute BASTA® (glufosinate ammonium) ingestion in an emergency room. The blood samples were collected during 79 hours (0, 6, 9, 13, 17, 21, 25, 29, 33, 41 and 79) and CSF was taken once after 12 hours.

Methods: 0.1 mL of blood was taken, and 0.2 mL of acetone was added, sonicated and centrifuged at 9000 rpm for 5 min. For the derivatization, 0.4 mL of pH 9.0 borate buffer and 0.05 mL of FMOC-Cl (9-fluorenylmethoxycarbonyl chloride) were added to the supernatant and vortexed for 1 min. In order to eliminate unreacted FMOC-Cl, 0.2 mL of ethyl acetate was added, vortexed and removed by centrifugation. The residues were filtered with 0.22 μm PDVF syringe filter and used for the LC-MS/MS analysis with electrospray ionization in negative ion mode. For the determination of 1-methoxy-2-propanol, blood and CSF samples were injected into GC/MS with SPME (solid phase microextraction) sampler. Protein precipitation using acetonitrile followed by LC-MS/MS analysis was performed for the determination of SLES.

Results: The estimated half-lives of glufosinate and 1-methoxy-2-propanol were 8.4 hours and 24.4 hours, respectively. The concentrations of glufosinate in blood after 13 hours and CSF after 12 hours were 85.7 and 5.1 mg/L, those of 1-methoxy-2-propanol were 49.8 and 55.7 mg/L, and those of SLES 2.9 and 8.0 mg/L, respectively.

Discussion/Conclusion: Analytical methods for glufosinate, 1-methoxy-2-propanol and SLES in blood and CSF were developed using LC-MS/MS and GC/MS. The concentrations of 1-methoxy-2-propanol in blood and CSF were similar at the collection time, and those of SLES were higher in CSF than blood, while those of glufosinate were lower in CSF than blood, suggesting that both 1-methoxy-2-propanol and SLES can pass the BBB but are slowly eliminated, indicating indirect CNS effect or delayed neurological complications.

P60: The Missing Step Towards Fully Automated LC-MS Screening of Urine Samples - Implementation of μSPE into the Analytical Workflow

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Background/Introduction: The great benefit of immunoassays in routine screening is the high degree of automation regarding sample preparation and reporting of results. In contrast, an appropriate sample preparation is crucial for LC-MS analysis of body fluids. Liquid-liquid extraction (LLE), solid phase extraction (SPE) or protein precipitation (PP) are often laborious but mandatory steps. Micro solid phase extraction (μSPE) offers workflow benefits that allow for fully automated routine LC-MS screening.

Objectives: Implementation of a μSPE step into existing LC-MS methods to achieve fully automated LC-MS screening of urine samples.

Methods: Compounds covering the retention time and mass range of the methods were chosen and analysis was performed using existing routine LC-MS^n and LC-QTOF-MS screening methods (Bruker Daltonik). Automated μSPE of 250 μl urine was carried out by a PAL RTC Sampler (CTC).

Three types of μSPE cartridges, C18 10 mg, C18 30 mg and DAU (ITSP Solutions Inc), were compared according to their S/N ratios at three different concentrations (25, 50, and 100 ng/ml) and reproducibility was tested by tenfold preparation of pooled urine. Recovery and matrix effects were evaluated using a protocol adapted from Matuszewski et al. (2003). Furthermore, ante and post mortem urine samples and spiked urine samples (100, 250 and 500 ng/ml) were analyzed using μSPE to compare the findings with the current LC-MS^n screening using PP.

Results: For a set of 139 compounds the identification rate of the LC-MS^n screening using μSPE could be improved from 74% to 84% (c_{Low}) and 90% to 96% (c_{High}) compared to PP. Due to higher sensitivity of the QTOF-MS all compounds could be detected even at low concentrations. Adjusting the cleaning steps of the μSPE sampler led to no detectable carry-over caused by the μSPE system.

Compared to C18-10, DAU cartridges led to higher S/N ratios for some of the analytes but overall findings were comparable. The C18-30 cartridge showed low absolute peak areas and was excluded from further studies.

The RSD for the extraction process ranged from 5.6 to 10.9% (C18-10) and 10.0 to 14.9% (DAU) at low concentrations. At the high concentration level, the DAU cartridge showed better RSD values (1.8 to 6.8%) than the C18-10 cartridge (4.3 to 11.2%).

Recovery ranged from 50 to 90% (C18-10) and 61 to 100% (DAU) at low concentrations, 37 to 100% (C18-10) and 63 to 100% (DAU) at medium concentrations and 32 to 93% (C18-10) and 53 to 91% (DAU) at high concentrations. The only compounds with unacceptable reproducibility and recovery were morphine-glucuronide and ecgonine methyl ester.

Comparable matrix effects could be observed for both cartridges with maximum ion suppression around 50 %. The μSPE-LC-MS^n screening results of urine samples from real casework were in good agreement with the results of the initial routine screening.

Conclusion/Discussion: Data from this evaluation proved the successful implementation of the μSPE step into the analytical workflow. LC-MS^n screening of fortified blank urine using μSPE led to similar or better results than the routine precipitation step. Regarding the results from recovery and reproducibility evaluation, the C18-10 cartridge seems to have issues retaining early eluting compounds. Further optimization of the protocol might increase the performance of the DAU cartridge but direct injection of the μSPE eluate limits the choice of solvents at the moment.

The chosen hardware for online μSPE could be successfully implemented in both screening workflows, LC-MS^n and LC-QTOF screening, enabling a completely automated LC-MS screening of urine samples from sample preparation to evaluation of data.

POSTER

P61: Determination of Varenicline in Human Urine by High Performance Liquid Chromatography- Electrospray Ionization -Tandem Mass Spectrometry

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Background/Introduction: Varenicline, under the trade name Chantix® in the US, is a FDA approved prescription drug for treatment of nicotine addiction. Since its release in 2006, more studies have conducted to investigate side effects and some other potential applications for this drug. A better quantitative analytical method is needed to aid those efforts.

Objective: Our goal was to develop a validated quick and simple liquid chromatography tandem mass spectrometry (LC-MS/MS) method to accurately measure varenicline concentration in human urine.

Methods: One-half ml of urine was used for the analysis. Since deuterated internal standard was not readily available we chose to use amphetamine-d_5 as the internal standard for varenicline. The urine was made basic (pH > 10) with NH_4OH and then extracted with n-butyl chloride: acetonitrile (4:1). The organic layer was collected, acidified with HCl, evaporated and reconstituted with 0.1% formic acid in water (0.1% FA). Varenicline and amphetamine-d_5 were detected in the mass spectrometer using selected-reaction monitoring with respective transitions of m/z 212 to 169 and 141 to 93. The calibration curve had eight concentration points ranging from 1.0 to 1000 ng/mL. Quality control (QC) samples were prepared at 3, 30 and 800 ng/mL. The LC column was a Varian 100 x 2.1 mm, 5 µm Inertsil ODS-3 column. The mobile phase was isocratic with 92% of 0.1% FA and 8% of acetonitrile.

Results: Specificity was determined from analysis of blank urine fortified with internal standard only (3 replicates) and with lower limit of quantitation (LLOQ) concentration (1.0 ng/mL) (1 replicate) in six different lots of urine. The primary evaluation was to compare mean peak area ratio of any signal at retention time of the analyte to its internal standard for each lot with the mean peak area ratio of the six LLOQ samples. Mean ratios relative to mean LLOQ ranged from 2.94 to 6.31 suggesting the LLOQ could be lowered if needed. Intra-run accuracy of the LLOQ was within 6.0% of target with intra-run precision within 11.3%. Intra- and inter-run precision and accuracy were also evaluated at three QC concentration levels. The intra-run accuracy was within 11.9% of target with intra-run precision within 5% (n=5). The inter-run accuracy was within 13.8% of target with inter-run precision within 7.6% (3 repetitions with n=5 at each repetition). Varenicline was stable in human urine for up to 18 hours at room temperature and after 3 freeze-thaw cycles. Stability in processed samples was 8 days in -20°C and 3 days on auto-sampler (10 °C) respectively. The mean extraction efficiency (n=5) was 71%.

Conclusion/Discussion: We have developed and validated a LC-MS/MS method for varenicline measurement in human urine. LLOQ was set at 1 ng/mL because we wanted to stress the high end of the analytical range (1000 ng/mL). Samples were analyzed successfully using this method in a randomized, double-blind phase II clinical trial study (Briones et al. Drug Alcohol Depend 189: 30-36, 2018)

P62: Confirmation and Quantitation of Seven Opioids in Human Urine by Liquid Chromatography Tandem Mass Spectrometry using RP-WAX Tips

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Background/Introduction: The Forensic Toxicology Drug Testing Laboratory (FTDTL) at Tripler Army Medical Center (TAMC), Oahu, HI has a monthly workload of 60,000 urine specimens received from service members in the Department of Defense (DOD) and the US Coast Guard. Among the drugs confirmed (1% positivity rate) at FTDTL TAMC, opiates and synthetic opioids make up 10-15% of the positive specimens reported every month.

Objectives: The goal of the study is to develop and validate a comprehensive opioid urine quantitation method using liquid chromatography tandem mass spectrometry (LC-MS/MS) for morphine, codeine, hydrocodone, hydromorphone, oxycodone, oxymorphone, and 6-acetylmorphine to replace four gas chromatography mass spectrometry (GC-MS) methods. This method was developed and validated according to the DOD requirements and Scientific Working Group on Toxicology guidelines. The DOD reporting cutoff, shown in table I, varies widely between the drugs in this assay, requiring a method that is sensitive and specific across the range of concentrations.

Compound	DOD Cutoff (ng/mL)
Morphine (MOR)	4000
Codeine (COD)	2000
Oxymorphone (OXMOR)	100
Oxycodone (OXCOD)	100
Hydromorphone (HYMOR)	100
Hydrocodone (HYCOD)	100
6-Acetylmorphine (6AM)	10

Table I. DoD reporting cutoff values.

Methods: Enzymatic hydrolysis was evaluated on morphine-3-β-D-glucuronide, codeine-6-β-D-glucuronide, oxymorphone-3-β-D-glucuronide, and hydromorphone-3-β-D-glucuronide using IMCSzyme (>50 kU/mL), IMCSzyme RT (>500 kU/mL), and KURA Biotec BGTurbo (>200kU/mL). Sample preparation utilized RP-WAX disposable pipette extraction (DPX Technologies, Columbia, SC) of 200 µL urine, with the option to fully automate on a Hamilton Microlab STAR series (Hamilton Company, Reno, NV) liquid handling system. Separation was performed on the Agilent 1290 Infinity Multisampler LC system with a Kinetex biphenyl column in 10 minutes using gradient elution with a flow rate of 0.4 mL/min, mobile phase A (10 mM ammonium formate in water, pH 5) and mobile phase B (0.1% formic acid in methanol). Transitions monitored on the Agilent 6460 triple quadrupole can be found in table II.

Compound	Prec Ion	Prod Ion 1	Prod Ion 2	Compound	Prec Ion	Prod Ion 1	Prod Ion 2
Morphine	286.2	165	152	Morphine-d6	292	153	152
Codeine	300.2	165	152	Codeine-d6	306	165	152
Oxymorphone	302.1	284	227	Oxymorphone-d3	305	287	230
Oxycodone	316.2	298	241	Oxycodone-d3	319	301	244
Hydromorphone	286.2	185	157	Hydromorphone-d6	292	185	157
Hydrocodone	300.2	199	128	Hydrocodone-d6	306	202	128
6-Acetylmorphine	328.1	211	165	6-Acetylmorphine-d6	334	211	165

Table II. MS-MS compound transitions

Results: Enzymatic hydrolysis replaced acid hydrolysis, achieving greater than 90% conversion of all glucuronides in 30 minutes; however, concentration of codeine-6-β-D-glucuronide was limited to 5,000 ng/mL. The Hamilton STAR systems were unable to heat the samples to the required 55°C in 96-well plates, decreasing conversion of codeine-6-β-D-glucuronide to approximately 80%. IMCSzyme RT and KURA Biotec BGTurbo were evaluated to allow for full automation of the method with greater than 90% conversion of all glucuronides around cutoff concentration. This was attained using IMCSzyme RT.

Linear ranges were 400 – 20,000 ng/mL for morphine, 200 – 20,000 ng/mL for codeine, 10-1,000 ng/mL for oxycodone/oxymorphone/ hydrocodone/ hydromorphone, and 1 – 100 ng/mL for 6-acetylmorphine. Intra and inter assay precision was <10% for all compounds at DOD cutoff, 40% of cutoff, and 125-150% of cutoff. Between 0.25 and 15% biases were achieved with known proficiency samples.

Conclusion/Discussion: A multi-analyte LC-MS/MS confirmatory method was developed using RP-WAX DPX tips. The advantage of this method is its applicability to either manual sample extraction, semi-automated, or fully automated approach.

POSTER

P64: Havoc in the Kitchen: Making to Do Some Baking, the Preparation of Matrix-Matched Cannabinoid Calibrators and Controls

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Background/Introduction: Use of cannabinoids in the United States has increased tremendously in the past decade. The biggest increase has been in marijuana edibles, or "medibles", that are typically packaged as candy or baked goods. These products contain delta-9-tetrahydrocannabinol (THC), and/or cannabidiol (CBD) Federally, the US Drug Enforcement Administration (DEA) has classified marijuana as a Schedule 1 substance. The only legal formulation of THC is Marinol®. In 2018, the FDA approved the use of CBD to treat two forms of epilepsy, and the Agriculture Improvement Act (Farm Bill) made hemp legal. These actions do not address the regulation of medibles, and there is no standardized method for preparation or analysis. The three most common medible matrices used are high fiber (brownies), high sugar (gummies), and high fat (dark chocolate).

Objective: To prepare matrix-matched high fiber, high sugar, and high fat quality control materials (QC) containing cannabinoids, and to develop and validate a method for their analysis.

Methods: The fiber matrix was prepared by following the directions on the brownie mix. Brownies were baked at 300°F, until done. The sugar matrix material was prepared using a mix of flavored gelatin, unflavored gelatin packet, and water. The gummy mix was heated in a microwave, then molded, and chilled. The fat matrix was prepared using cacao butter, cocoa powder, oil, honey and vanilla. Several dark chocolate recipes were evaluated for ease of preparation and palatability. The ingredients were melted in a double boiler and tempered the following day. Cannabinoid concentrations in stability QC were prepared at 5 and 10 mg servings. Calibration materials were prepared by fortifying 25mg of each drug-free matrix at 0.8, 1.6, 4, 8, 16, 40, and 80 mcg/g THC, CBD, and CBN. Method validation QC was prepared by fortifying the drug-free matrix before preparation (i.e. baking, mixing, and tempering) at 0.8, 2.4, 24, and 60 mcg/g THC, CBD, and CBN. The QuEChERS sample preparation method involved a modified Phenomenex® roQ™ application note. Briefly, 1.5mL of LCMS-grade water and 1.5mL of LC-MS grade acetonitrile were added to 25mg of sample. Samples equilibrated at room temperature for one hour, then vortexed, and 0.65g of QuEChERS salt was added. The samples were shaken in a Biotage® BeadRuptor® 24 for 30seconds at 5cm/s, then centrifuged at 3500rpm for 10minutes. One milliliter of the upper ACN layer was transferred to a Phenomenex® roQ™ dSPE tube, vortexed for 1 minute and centrifuged at 8000g for 5minutes. The upper layer was transferred to a glass insert 96well plate and analyzed via UPLC-MS/MS with an injection volume of 5mcL.

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Results: All calibration curves for THC, CBN and CBD had r^2 >0.996. The bias ±20% with CVs <15% were determined for all controls. Cannabinoids in the matrices were stable at room temperature, 5℃, -15℃, -70℃ for up to six months. Sufficient mixing is necessary to ensure homogeneity. Brownies are baked in mini-muffin or brownie bite tins at <300°F to maintain the stability and reduce baking time. Gummies need minimal heating to dissolve the sugar matrix, and are molded soon after dissolution. Chocolate must be tempered to ensure homogeneity.

Conclusion/Discussion: The lack of commercially available matrix-matched materials makes these QC materials necessary. The QC materials are easy to prepare, but care must be taken to ensure homogeneity and stability of the cannabinoids in each matrix. The modified Phenomenex® roQ™ QuEChERS method was robust and reliable for the analysis of THC, CBD, and CBN.

P65: Accuracy by Accident in THC-COOH Measurement

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Background/Introduction: The measurement of marijuana consumption in humans is generally performed as the 11-nor-9-car-boxy-tetrahydrocannabinol (THC-COOH) metabolite in urine. This typically involves hydrolysis of conjugated forms to yield the total metabolite. The dichotomy between the intended measurand and the physiologically produced/conjugated species presents a host of variables, notably that of well-described adsorptive loss of THC-COOH that differs from that of THC-COOH-Glucuronide. As such, this work will investigate whether reference materials are truly commutable for the analysis of THC-COOH in human urine

During development of an automated THC-COOH by LC-MS/MS assay, THC-COOH recoveries from authentic and contrived specimens differed dramatically based on matrix as well as sample treatment. Variations in recovery were experimentally explored to elucidate the causes based on perceived solubility of discrete metabolites, metabolite content relative to the final measured form, specimen handling, analytical procedures and dilutional recoveries. These experiments were performed on positive human samples, proficiency test specimens, standard reference materials from the National Institute of Standards and Technology (NIST) and modified matrices in order to establish trueness and accuracy in the measurement of total THC-COOH in human urine.

Objectives: Experimentally demonstrate variations in recovery of total THC-COOH as a function of calibration matrix and extraction.

Methods: Urine specimens tested as both positive and negative for marijuana metabolites via GC-MS were collected after in-house testing and de-identified. Negative specimens, a THC-COOH-soluble matrix (40% methanol in negative urine) and methanol were fortified with free THC-COOH to generate calibration standards. NIST vials were separated into 2 groups, one of which was prepared via instructions (H_2O), the other reconstituted with 40% methanol in H_2O. Samples were sub-aliquoted to silanized glass tubes. Calibration standards were prepared in 40% methanol in urine; quality controls were prepared by pooling positive urine specimens and diluting to target values of 7.5 ng/mL and 18 ng/mL. Control values were assigned by replicate analysis (n=20).

All samples were submitted to a battery of evaluations including multi-tube transfers, multi-pipetting-tip exposures, dilutional linearity, tube pretreatments and organic dilutions. Samples were hydrolyzed after a basic solution and internal standard addition via automated liquid handling (100 µL sample, 30 µL 2N NaOH, 50 µL isotopically-labeled internal standard in methanol) at 55°C for 30 minutes. The liquid handlers were programmed to mitigate observed losses due to adsorption in sample manipulation. After hydrolysis, samples were buffered to a pH of approximately 4 and were analyzed via LC-MS/MS.

All samples were injected on a Waters Acquity system using Type I water with 0.1% formic acid as mobile phase A and methanol as mobile phase B through an Acquity PFP, 50x2.1 mm column with a Sciex 5500 triple quadrupole mass spectrometer detection system. Four ion transitions were monitored for THC-COOH; 3 transitions were monitored for the isotopic internal standard. Data were reduced in Indigo Biosystems Ascent software.

Results: The automated assay for THC-COOH assay showed CV%'s of 2.4% for an 18 ng/mL control (n=40) and 3.3% for a 7.5 ng/mL control. A 40% methanol in negative urine calibration curve (5 to 1000 ng/mL) demonstrated equivalency to 100% methanol matrix as determined by Deming regressions (n=6, mean slope =0.99, r = 0.9993). Negative urine fortified with THC-COOH demonstrated poor accuracy with 40% methanol in urine, though good correlation (n=6, mean slope 0.69, r=0.9981). NIST specimens diluted as prescribed (with 20 mL H_2O) yielded a mean -24% bias (n-6); the same NIST specimens reconstituted with 40% methanol in urine yielded acceptable bias (n= 6; bias = 0.34%).

 Human specimens fortified with THC-COOH showed a range of negative biases compared to a methanol enriched calibration curve (-42% to -67%). Human urines containing authentic metabolites indicated a lesser range of disagreement (-1.7% to -17%).

Conclusion/Discussion: Proficiency tests and standard reference materials from the National Institute of Standards and Technology only assess target values for commutable materials based on the measurement of THC-COOH in human urine (or urine-like matrices). This presents a particular problem in that calibration or QC materials prepared using free THC-COOH are not equivalent to human-derived metabolites in regards to solubility; thus pre-analytical variables of THC-COOH and THC-COOH-Glucuronide are inherently disparate, despite the 2 molecules being considered one and the same in many assays. Variations in recovery based on the calibration matrix and calibration materials have been experimentally demonstrated and should be considered in all test evaluations.

POSTER

P66: Certification of Phosphatidylethanol Solution Standards for Long-term Alcohol Abuse Testing

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Background/Introduction: Phosphatidylethanols (PEths) are biomarkers associated with ethanol consumption; PEth quantitation can indicate amount of ethanol consumed as well as suggest a pattern of consumption such as social drinking versus binge drinking. Advantages of PEth testing over traditional blood-alcohol tests are that they are direct biomarkers only associated with alcohol consumption and have a longer detection window. PEth testing is performed on whole blood samples with quantitative liquid chromatography/tandem mass spectrometry (LC/MS/MS). PEth 16:0/18:1 and PEth 16:0/18:2 are two of the most prevalent homologues quantified with PEth testing. The accuracy and reproducibility of the assay is dependent upon the accuracy and reproducibility of calibrator preparation, among other factors. The use of Certified Reference Materials (CRMs) as ampouled solutions to prepare calibrators and controls can improve assay accuracy as well as decrease preparation time.

Objectives: Develop a stable formulation of PEth 16:0/18:1 and PEth 16:0/18:2 in solution standard format to prepare calibrators and controls for PEth testing. Develop a stable isotope labeled PEth 16:0/18:1-D_5 solution standard for use as an internal standard. Certify the solution standards as CRMs to provide increased accuracy and reproducibility of PEth LC/MS/MS assays. Demonstrate stability of the solution standard CRMs with real-time and accelerated stability testing.

Methods: Purity assignment of raw materials was performed by quantitative ^{1}H-NMR (qNMR). Solution standards were prepared gravimetrically and dispensed and flame-sealed in amber ampoules under an inert atmosphere. Concentration and homogeneity verification were performed by LC/MS/MS assay against an independently prepared calibration curve. The analyzed concentration accuracy was within 5% of target and %CV for ampoules pulled across the lot was less than 3%. The assay method was a gradient reversed phase LC method on a C18 column. Accelerated stability testing of PEth 16:0/18:1 and PEth 16:0/18:2 was performed on ampoules stored at -70 °C, -15 °C, 4 °C, 21 °C, and 40 °C for up to 4 weeks and analyzed for purity by LC/MS.

Results: PEth 16:0/18:1 and PEth 16:0/18:2 were prepared as single-component solution standards at a concentration of 1 mg/mL in methanol. PEth 16:0/18:1-D_5 was prepared at a concentration of 100 µg/mL in methanol. PEth 16:0/18:1 and PEth 16:0/18:2 CRM concentration and homogeneity were verified by LC/MS/MS assay against independently prepared calibration curves. Stability testing indicates stability at the recommended storage condition with no decrease in purity noted at -70 C and -15 C storage; real-time stability testing is on-going. Stability testing of PEth 16:0/18:1-D_5 is on-going.

Conclusion/Discussion: Certified Reference Materials of PEth 16:0/18:1 and PEth 16:0/18:2 have been prepared and certified in solution standard format for use in PEth testing to prepare LC/MS/MS assay calibrators and controls. The CRMs have demonstrated stability under stressed conditions with real-time stability studies on-going. A CRM of PEth 16:0/18:1-D_5 has been developed for use as an internal standard and is currently undergoing homogeneity, concentration, and stability testing. These CRMs will provide improved accuracy and reproducibility for PEth LC/MS/MS assays by providing traceable, accuracy based certified calibration materials for quantitation.

POSTER

P67: LC-MS/MS Method Development and Analysis of Tricyclic Antidepressants in Human Plasma

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Background/Introduction: Depression has become one of the most prominent problems in society, disrupting both personal and social lives. Tricyclic antidepressants (TCA's) were first developed in the 1950's and became some of the leading anti-depressant medications on the market later that decade. Today, newer anti-depressants have risen to the forefront, being safer and having a lower side-effect probability. Nonetheless, TCA's continue to be prescribed for severe depression, especially in cases where the newer anti-depressants have failed. However, TCA's are still highly potent, the toxicity associated with these compounds cannot be ignored. They have considerable cardiovascular and neurological toxicity, and in the event of an overdose, may lead to death within an hour.

Objectives: In an effort to identify the concentration of these TCA's in individuals, an LC-MS/MS method was developed and validated for the analysis of amitriptyline, desipramine, imipramine, and nortriptyline in human plasma samples.

Methods: The method was developed and validated on two different LC-MS/MS instruments, ABSciex QTRAP 3200 and ABSciex QTRAP 4500. Two specific MRM transitions for amitriptyline, desipramine, imipramine, and nortriptyline, and two specific MRM transitions for amitriptyline-d_3, desipramine-d_3, imipramine-d_3, and nortriptyline-d_3 internal standards, were monitored for each compound for maximum selectivity and sensitivity. Separation was achieved on a Synergi Hydro-RP column (150 x 3.00 mm; 4 µm; 80 Å), and data acquisition and processing were performed with Analyst™ 1.6.3 software. The extraction procedure was developed to be rapid and simple, involving crashing the plasma with cold acetonitrile (ACN), filtering through a 0.2 µ polypropylene (PP) filter, evaporation, and reconstitution.

Results: The limit of detection (LOD) and limit of quantitation (LOQ) were identified to be 1 ng/mL, and the upper limit of linearity (ULOL) was determined to be 400 ng/mL. On the ABSciex QTRAP 3200 instrument, the coefficient of variance (%CV) for the low control (40 ng/mL) for within the batch varied from 1.0%-8.5% for all compounds, while the %CV from batch to batch for the low control ranged 3.3%-11.4%; in addition, the %CV for the high control (120 ng/mL) for within the batch ranged from 0.5%-2.8%, while the %CV from batch to batch for the high control ranged 1.0%-5.0%. On the ABSciex QTRAP 4500 instrument, the %CV for the low control (40 ng/mL) for within the batch varied from 1.3%-5.3% for all compounds, while the %CV from batch to batch for the low control ranged 0.9%-7.8%; in addition, the %CV for the high control (120 ng/mL) for within the batch ranged from 1.4%-3.6%, while the %CV from batch to batch for the high control ranged from 0.2%-5.1%. The accuracies for the 40 ng/mL control on the 3200 instrument ranged from 89% to 97%, and at the 120 ng/mL level the accuracies varied from 95% to 99%; the accuracies for the 40 ng/mL control on the 4500 instrument ranged from 92% to 99%, and at the 120 ng/mL level the accuracies varied from 95% to 100%. The developed method was applied to more than 350 human plasma samples. Matrix effect and drug interference studies were carried out, and neither matrix effect nor drug interference was observed.

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Conclusion/Discussion: The developed and validated LC-MS/MS method was robust, reproducible, and highly sensitive for the determination of low levels of the TCA's in human plasma samples.

P68: Screening and Quantitation of Δ^9-Tetrahydrocannabinol (Δ^9-THC) and Cannabidiol (CBD) from Different Commercial Products using GC-FID

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Background/Introduction: Over the past few years, hemp products have been marketed for their supposed medicinal and therapeutic effects. The number of commercially available hemp products on the market has dramatically increased, in response to the increased demand for cannabidiol (CBD), a cannabis constituent without the psychoactive effects known for Δ^9-Tetrahydrocannabinol (Δ^9-THC), but with numerous purported beneficial health effects.

Objectives: In order to better understand the Δ^9-THC and CBD content of commercially available hemp products, a previously developed and validated GC-FID method was used to screen and quantitate Δ^9-THC and CBD in twenty-three different hemp products.

Methods: The previously developed and validated GC-FID method was run in the split mode with a total run time of 17.58 minutes. Separation was achieved on a DB-1ms capillary column, with dimensions of 15.0 m x 250 µm x 0.25 µm; the makeup gas for the flame ionization detector (FID) was helium, at a flow rate of 27 mL/min.

Results: A total of twenty three commercially available hemp products were analyzed for the concentrations of Δ^9-THC and CBD. The hemp products included honey sticks, vape additives, hemp oil, syrups, candy, hemp, CBD oil, pure CBD, and creams. Most of the products contained less than 0.3% Δ^9-THC and 0.5% CBD. Three products were identified to have concentrations of Δ^9-THC greater than 0.3%, with one product containing a very high concentration of Δ^9-THC: approximately 45.1%. Five products were determined to have CBD concentrations ranging from 1% to 5%. Two products were determined to have very high concentrations of CBD, with one product containing approximately 41.8% CBD and the other containing 100% CBD. Three products contained < 0.10% of either CBD or Δ^9-THC but reported by the marketer as giving strong high. These are currently being investigated for content of synthetic cannabinoids.

Conclusion/Discussion: The previously developed GC-FID method proved to be sensitive for use to detect low levels of both Δ^9-THC and CBD in commercial hemp products and can be used for screening and quantitating the concentrations of both Δ^9-THC and CBD in a wide variety of products.

POSTER

P69: In-house Validation of Randox© AB-PINACA Enzyme-Linked Immunosorbent Assay (ELISA) Kits and Cross-Reactivity Evaluation of Emerging Synthetic Cannabinoids

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Background/Introduction: In-house validation is essential to the implementation of immunoassay into routine laboratory testing to verify the performance provided by the manufacturer and to determine the specificity. According to the Drug Enforcement Agency, FUB-AMB and 5-fluoro-MDMB-PINACA were the most prevalently encountered synthetic cannabinoids in 2018.   These statistics were supported through testing performed within the District of Columbia. In addition to FUB-AMB and 5-fluoro-MDMB-PINACA, ADB-FUBINACA, AB-FUBINACA, MMB-CHMICA, 5-fluoro-AMB, and MDMB-FUBINACA were also evaluated for cross-reactivity.

Objectives: The goal was to validate the Randox© AB-PINACA Enzyme-Linked Immunosorbent Assay (ELISA) kit based on SWGTOX and AAFS Standards Board (ASB) immunoassay validation criteria. In order to achieve the goal, the following parameters were evaluated: limit of detection, hook effect, carryover, and precision. Additionally, cross-reactivity was established for 7 synthetic cannabinoids that are commonly identified in Washington, DC.

Methods: Four hundred microliters of blood was diluted by 1600 microliters of buffer. Fifty microliters of diluted sample was pipetted into coated wells of the AB-PINACA microtiter plate followed by 75 microliters of conjugate. The plate incubated for an hour, then washed and dried six times. One hundred twenty-five microliters of substrate solution was added to each well and incubated for 20 minutes. One hundred microliters of stop solution was added to each well and the optical density was measured.

Results: Replicate data from within day and between day studies supported a reproducible limit of detection of 2 ng/mL (CV = 9.39%). Therefore, the decision point was 2 ng/mL and the high and low calibrators are 4 ng/mL and 1 ng/mL, respectively. Fortified blood samples of 100 ng/mL of AB-PINACA 5-Pentanoic acid demonstrated no increase in absorbance; therefore, demonstrating no hook effect up to 100 ng/mL. A blank blood sample was run immediately following one set of triplicates of 100 ng/mL samples and resulted in a negative value, ruling out the possibility of carryover for samples at concentrations up to 100 ng/mL. The intra-plate precision replicates at various concentrations for AB-PINACA 5-Pentanoic Acid had an overall %CV less than 10%. The inter-plate precision replicates at various concentrations for AB-PINACA 5-Pentanoic Acid had a %CV less than 14%. The %CV of each concentration of AB-PINACA 5-Pentanoic acid normalized against the negative was less than 10%. ADB-FUBINACA demonstrated 31% cross reactivity at 0.5 ng/mL. At 10 ng/mL, ADB-FUBINACA demonstrated 284% cross reactivity. AB-FUBINACA demonstrated 4.5% cross reactivity at 0.5ng/mL and at most 28% cross reactivity at 5 ng/mL. FUB-AMB, MMB-CHMICA, 5-fluoro-AMB, MDMB-FUBINACA and 5-fluoro-MDMB-PINACA resulted in <1% cross reactivity at all concentrations.

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Conclusion/Discussion: The lab was able to validate an in-house method for the Randox© AB-PINACA ELISA kits and determine limit of detection, hook effect, carryover, precision, and specificity. The expectation was that the %CV of AB-PINACA 5-Pentanoic acid standard should be less than 20% and it was consistently below 15%. While determining specificity, AB-FUBINACA and ADB-FUBINACA exhibited significant cross-reactivity with the kit. Both responded at concentrations as low as 0.5 ng/mL. The lab was unable to meet the suggested SWGTOX guideline of the mean of the concentration pools of AB-PINACA 5-Pentanoic Acid optical density values ± two standard deviations should not overlap at each concentration for the decision point to be valid. However, the ASB states the grand mean of ± two standard deviations of the low and high concentration pools of AB-PINACA 5-Pentanoic acid should not overlap with the grand mean of the decision point. The in-house validation met the ASB criteria.

P70: Ultra-high-performance Liquid Chromatograph Tandem Mass Spectrometer (UHPLC-MS/MS) Method for the Analysis of DOI in Mouse Blood and Brain with Corresponding Head-twitch Response (HTR)

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Background/Introduction: 2,5-Dimethoxy-4-iodoamphetamine (DOI) is a serotonergic psychedelic 5-HT$_{2A}$ receptor agonist first synthesized by Alexander Shulgin. The 5-HT$_{2A}$ receptor is a target of serotonergic psychedelic drugs and mediates, at least partly, the action of many antipsychotic drugs. DOI is a psychedelic in a class of 5-HT$_{2A}$ receptor agonists that includes lysergic acid diethylamide (LSD), psilocybin, N,N-dimethyltryptamine, mescaline, 2,5-dimethoxy-4-bromophenethylamine (2C-B) and 2-(4-chloro-2,5-dimethoxyphenyl)-N-(2-methoxybenzyl)ethanamine (25I-NBOMe). Many of these psychedelic drugs cause remarkably similar effects, despite their different chemical structures. These 5-HT$_{2A}$ receptor agonists produce a behavioral signature know as head-twitch response (HTR) in rodents. The HTR was first reported in 1956 by Keller and Umbreit following intravenous administration of LSD to mice and consists of a rapid and violent head shaking that occurs at a much lower frequency in vehicle-injected mice. Numerous studies have demonstrated dose-dependent effects of DOI in rats and mice, an increase in the doses results in increases in HTRs. Presented are blood and brain concentrations, and corresponding HTR for Intraperitoneal (*i.p.*) injection of 1 mg/kg DOI along with the ultra-high-performance liquid chromatograph tandem mass spectrometer (UHPLC-MS/MS) method used for the DOI analysis.

Objectives: To develop a UHPLC-MS/MS method for the analysis of DOI in mouse blood and brain tissue and compare the determined concentrations and elimination rates to that of the HTR.

Methods: HTR was measured in the mice by placing a small neodymium magnet on the top of the cranium. After, mice were given 1 mg/kg DOI or vehicle (saline) *i.p*, they were placed in a coil where the movement of the head was converted to voltage signal by electromagnetic induction and recorded. A dedicated algorithm performed the automated quantification of HTR events.

A separate batch of mice were given 1 mg/kg DOI *i.p*. From different animals, blood and brain tissue samples were collected in quadruplicate at 0, 0.5, 1, 2, 4 and 24 hours. Brain tissue was homogenized (1:3 tissue: water). The method used for analysis was validated for linearity, precision, accuracy, carryover, specificity, matrix effects, and stability in mouse blood and brain. A seven-point calibration curve (1-500 ng/mL DOI), quality controls, and samples with internal standard (10 ng/mL 2-CB) added were extracted using sodium hydroxide and hexane/ethyl acetate (9:1). The organic solvent was evaporated. The samples were then reconstituted in mobile phase and analyzed on a Shimadzu UHPLC system (Kyoto, Japan) attached to a Sciex 6500+ QTRAP system (Sciex, Ontario, Canada). Chromatographic separation was performed on a Hypersil Gold C8, 100×2.1 mm, 3 μm column (Thermo Scientific, Waltham, MA, USA). The mobile phase consisted of A: 10 mM ammonium acetate and 0.1% formic acid and B: Acetonitrile. The following gradient was applied: 0.00–0.10 min, 20% B, a linear gradient to 70% B at 3.00 min, hold for 0.90 min, then return to 20% B at 4.0 min. The following transition ions (m/z) were monitored in MRM mode with their corresponding collection energies (eV) in parentheses: DOI: 322 >305 (15) and 322>271 (28); and 2-CB: 260 > 243 (15) and 260 > 228 (28). The total run time for the analytical method was 5 min.

Results: The developed UHPLC-MS/MS method was used to determine mouse blood and brain concentrations. Corresponding to the HTR time-course maximal concentration were reached in the first 30 min, blood 76±12 mg/mL and brain 466±136 mg/g. The calculated half-lives of DOI were similar for HTR, blood and brain.

Conclusion/Discussion: The method for the analysis was successful in quantifying DOI in mouse blood and brain. HTR was shown to have a positive correlation to the blood and brain concentrations.

POSTER

P71: Comparing the Clinical Characteristics in Traditional Substance-Abusing Patients with New Psychoactive Substance-Abusing Patients in the Emergency Department

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Background/Introduction: The growing number of cases and the severity of the effects of NPS abuse have elicited substantial concern from healthcare providers and legal authorities. There is relatively little information on the analysis or clinical effects of illicit substances in East- and Southeast Asia although many illicit substances including NPS are produced in these regions.

Objectives: Our objective was to report acute complications or toxicity after analytically confirming substance abuse in patients presenting to the emergency department (ED) in Taiwan. We compared the clinical manifestations in patients of traditional substance abuse with the patients of NPS abuse.

Methods: We performed a retrospective study between May 2017 and August 2018 on patients presenting to the ED with acute complications and positive urine drug analysis by liquid chromatography tandem-mass spectrometry (LC-MS/MS). All urine sample were analyzed by the immunoassay (Alere Triage® TOX Drug Screen; Alere, San Diego, CA, USA, including acetaminophen, amphetamine/methamphetamine, barbiturates, benzodiazepines, cocaine, methadone, opiate, phencyclidine, tetrahydrocannabinol and tricyclic antidepressants) and LC-MS/MS analysis.

Results: A total of 1,069 urine samples were analyzed for all patients presenting to the EDs with suspected or declared drug-related toxicity or complications. Two hundred eleven samples (19.7%) were tested positive for more than one illicit substance by LC-MS/MS. From the 211 cases, 203 cases older than 10 years old were included, whereas eight cases younger than 10 years old were excluded because four cases were victims of child abuse and the other four were cases of neonatal drug abstinence syndrome. Traditional illicit substances (morphine analogue except methadone, amphetamine/methamphetamine, cocaine, cannabis, and MDMA: traditional group) were found in 138 (68%) samples, and NPS alone (NPS group) in 41 (20.2%) samples and NPS combined with traditional illicit substances (combined group) in 24 (11.8%) samples. Methamphetamine was the most common illicit substance (136/203; 67.9%). The most common NPS were ketamine (44/203; 21.7%) and followed by synthetic cathinones (30/203; 14.8%) with 4-Methyl-α-ethylaminopentiophenone (13/203,6.4%) and, mephedrone (9/203,4.4 %) and methylone (9/203,4.4%) being the most common. Polysubstance abuse was more common in the NPS group than the traditional illegal substance group (P < 0.001). Most patients were men (78.3%), and the average age was lower in the NPS group (*P* < 0.001). In the NPS group, >70% of the patients were <30 y/o and more than a quarter were ≤20 y. The average age of the cathinone cases was comparatively lower compared to other abused substance (*P* < 0.001). Cathinone abuse may be more popular in the young people. of 203 cases with more than one illicit substances detected by LC-MS/MS, 55 cases (27.0%) were self-reported. The cultural factors prevent our patients from self-reports of substance abuse because most cases visited our ED accompanied by their family especially parents. There is social pressure on the patients to declare substance abuse. Approximately 60% of 203 cases were positive for methamphetamine/amphetamine, morphine, and cannabis according to the urine immunoassays. Although the chemical structures of cathinones are similar to the amphetamine, 92.0% (23/25) of the cathinone abused cases without the combination with methamphetamine abuse were negative according to the immunoassay

Conclusion/Discussion: Our study provided by LC-MS/MS analysis data on acute illicit substance toxicity or complications at the ED in Taiwan. We did not include the cases who had symptoms, but no positive detected by LC-MS/MS. Our results may have underestimated the number of substance abusers in our population. More than one third cases were NPS abusers in our study. Young adults and polysubstance abusers were more commonly the NPS group. Ketamine and cathinone were the most common NPS. Self-reports were not usually obtained, and the positive detection rates were low by immunoassay. Therefore, differential diagnosis was difficult and analytical confirmation is necessary.

POSTER

P72: Long-Term Stability of Novel Synthetic Opioids in Blood

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Introduction: Recently, there has been an increase in overdose deaths due to novel synthetic opioids (NSO). Fatalities have been reported for AH-7921, U-47700, U-49900, and MT-45. Due to backlogs experienced by many forensic laboratories, samples containing these analytes could be stored for an unknown amount of time before analysis; therefore, it is important to understand their stability in a variety of storage conditions.

Objectives: To investigate the stability of AH-7921, U-47700, U-49900, U-50488, MT-45, W-15, and W-18 in blood at various temperatures over 36 weeks.

Methods: Blood (100 mL) preserved with sodium oxalate was fortified at a low quality control (LQC) concentration (0.75 ng/mL for AH-7921, U-47700, U-49900, U-50488, MT-45 and W-15/ 2.5 ng/mL for W-18) and a high quality control (HQC) concentration (80 ng/mL for all analytes). The LQC and HQC fortified blood were equally disbursed into empty vacutainer tubes and placed into appropriate temperature settings: frozen (-20°C), refrigerated (-4°C), room temperature (~25°C), and elevated temperature (35°C). A total of 11 time points were analyzed in duplicate in this study (T_0, 3 days, 1 weeks, 2 weeks, 3 weeks, 4 weeks, 8 weeks, 12 weeks, 16 weeks, 31 weeks, and 36 weeks). Samples were extracted and analyzed using a previously validated method [Lowry, *Forensic Toxicol,* 2019]. An additional protein precipitation step was added to reduce solvent usage and time. Blood (0.5 mL) was fortified with internal standard then ice cold acetonitrile (1 mL) was added. Samples were then centrifuged (2360 x g) for 5 mins and decanted in a new tube. SPEware PolyChrom ClinII 3 cc (35 mg) (Baldwin Park, CA) solid phase extraction (SPE) cartridges were used for extraction. Briefly, samples were buffered (100 mM phosphate buffer, pH 6), loaded onto cartridges, then washed, and dried. Acidic and basic drugs were eluted with ethyl acetate and dichloromethane: isopropyl alcohol with 5% ammonium hydroxide, respectively. Combined elution fractions were dried under nitrogen and reconstituted in 0.25 mL of mobile phase. Samples were analyzed using an Agilent 1290 Infinity II liquid chromatograph coupled to an Agilent 6470 triple quadruple mass spectrometer. Stability was determined as the %target of T_0. Analytes were deemed stable if they were within ±20% of target concentration.

Results: Novel synthetic opioids were generally stable over the 36-week period (66%-118%) at both concentrations when blood samples were stored in the refrigerator or freezer. Most analytes were stable for at least 2 weeks at room temperature (77%-120%) at both concentrations, with the exception of AH-7921 and U-50488. At the elevated temperature, analytes were generally stable for at least 1 week (75%-109%). No differences were observed between analyte stability in the different concentrations.

Discussion/Conclusion: This study has determined the stability of several NSO at various temperatures over a 36-week period. These results reflect the forensic significance of keeping samples stored at proper temperatures. The results showed minimal effect on stability at the elevated temperature during the first week, indicating that these analytes would be stable in the event of improper transport/handling within this timeframe. However, blood samples suspected to contain synthetic opioids should be stored refrigerated or frozen, when possible, in order to preserve analyte stability, even at low concentrations.

Keywords: NSO, U-series, W-series, Stability

POSTER

P73: Comprehensive Screening of Traditional and Novel Psychoactive Substances in Postmortem Dried Blood Spots Specimens by UHPLC-QToF-MS/MS

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Background/Introduction: Dried Blood Spots (DBS) are an emerging sampling technology in Forensic Toxicology. DBS require less sample volume for analysis, as well as optimizing the processes of sample storage and transportation without special conditions. With the large number of novel psychoactive substances (NPS) and the need for rapid toxicological analysis, DBS is an interesting and promising technique for screening and quantitation of NPS by forensic laboratories.

Objectives: To develop a comprehensive screening method for traditional and novel psychoactive substances (such as cocaine, methamphetamine, MDMA, ketamine, LSD, NBOMes, 3-MeO-PCP, synthetic cathinones, benzodiazepines and designer opioids) in DBS using liquid chromatography/quadrupole-time-of-flight mass spectrometry.

Methods: 50 µL of blood was spotted on a pre-punched 1-cm diameter Whatman® 903 Protein Saver Cards and dried for at least 2h at room temperature, protected from light. Two whole spots were used and extracted using 1 mL of methanol, 50 µL of borate buffer (pH 10.4), and incubation at 25°C for 15 minutes under agitation. The extracts were dried, reconstituted with 100 µL of initial condition mobile phase, 90:10 0.1% 5 mM ammonium formate in water (pH 3)/0.1% formic acid in acetonitrile, and centrifuged for 5 minutes at 10,000 rpm. 10 µL of the extract was injected into the UPLC-MS/MS. Analysis was performed using a Waters Acquity I-class UPLC® coupled to a Xevo® G2-S QTOF. The chromatographic separation was performed using an Acquity UPLC® BEH C18 (2.1 mm x 150 mm, 1.8 um) column at 50°C and a flow rate of 0.4 mL/min. The total run time was 15 min. Method validation was performed assessing the limit of detection, extraction recovery, matrix effects and post-processed stability. The method was applied to the analysis of 68 specimens previously screened positive for at least one drug in the scope of this method by NMS Labs between 2016 and 2017. Positive criteria for identification were mass error less than 5 ppm, retention time shift lower than 0.25 min and peak area higher than 800.

Results: The cut-offs for all compounds ranged between 0.5 and 20 ng/mL. Average extraction efficiencies were higher than 70% for all compounds, except for heroin (69.9%) and LSD (69.7%). Matrix effects ranged from -52.9% for etizolam to 149% for LSD. No carryover was observed up to 500 ng/mL. No interferences from internal standards or 27 common therapeutic drugs or pesticides were observed. The compounds presented acceptable post-processed stability up to 48h in the autosampler at room temperature.

The method was then applied to the re-analysis of authentic postmortem and DUID specimens, previously analyzed using 0.5 mL sample volume. The results are expressed as positive DBS samples/positive blood samples: cocaine (13/14), fentanyl (13/16), methamphetamine (11/13), alprazolam (10/17), U-47700 (12/13), furanyl fentanyl (16/16), methoxyacetylfentanyl (6/6), N-ethyl-pentylone (3/3), p-fluoroisobutyrylfentanyl (FiBF) (8/9), acetylfentanyl (1/3) and acrylfentanyl (0/1). Our results suggest that there is a good correlation between the toxicological findings in blood and in DBS, for different postmortem blood matrices. For the drugs detected by traditional means of analysis but not detected in DBS, concentrations lower than the LOD or a possible degradation still in blood occurred over the time are some explanations.

Conclusion/Discussion: DBS were successfully applied for the identification of psychoactive drugs with different chemical properties, using a one-step, fast and easy to perform sample preparation method. Our findings corroborate that the DBS approach can be used for postmortem toxicological investigation of different classes of drugs, as an alternative to liquid blood, requiring less sample and reducing the sample preparation time.

POSTER

P74: Determination of MDPV and its Major Metabolites in Rat Brain by Liquid Chromatography-Mass Spectrometry

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Background/Introduction: MDPV (3,4-methylenedioxypyrovalerone) is a novel drug belonging to the synthetic cathinone family. These new substances mimic the effects of illicit stimulants such as cocaine, amphetamines, and ecstasy (3,4-methylenedioxy-methamphetamine). They possess a high potential for the development of serious problems, including psychosis, hallucinations, agitation, tachycardia, hypertension, hyperthermia, and even death. Although the popularity of these drugs has dramatically risen, limited data about their pharmacology are available. The development of analytical methods in different biological matrices is critical to support pharmacokinetic and pharmacodynamic studies of these novel psychoactive substances. Previous studies involving MDPV have identified two main metabolites, 4-hydroxy-3-methoxy-pyrovalerone (4-OH-3-MeO-PV) and 3,4-dihydroxy-pyrovalerone (3,4-catechol-PV), and have quantified these in human and rat plasma. However, publications involving methodologies in brain are scarce.

Objectives: To develop and validate a liquid-chromatography tandem mass-spectrometry (LC-MSMS) method for the determination of MDPV and its metabolites 4-OH-3-MeO-PV and 3,4-catechol-PV in rat brain samples.

Methods: Fifty-mg of rat brain were weighed and suspended in 500μL of 3% 250mM sodium metabisulfite (SMBS) and 3% 250mM ethylenediaminetetraacetic acid (EDTA) in 0.01M formic acid into 2mL homogenizer tubes containing 1.4mm ceramic beads. After homogenization in the bead mill, the samples were incubated with 10μL β-glucuronidase BG100® from Red Abalone (Kura Biotec) at 50°C 1h to perform hydrolysis. After adding 50μL of 5M ammonium hydroxide, the samples were submitted to supported liquid extraction and eluted by 2x900μL of ethyl acetate. The eluent was acidified with HCl in methanol (1:99) before evaporation. After reconstitution with 200μL of 0.1% formic acid in water, 20μL were injected into the LC-MSMS (LCMS-8050, Shimadzu) with electrospray in positive mode. The chromatographic separation was performed on a Synergi Polar reversed-phase column 2.1x100mm, 2.5μm (Phenomenex), employing a gradient of 0.1% formic acid in water and in acetonitrile, and 2 multiple reaction monitoring (MRM) transitions were monitored for all compounds. The method was validated evaluating linearity (n=5), limits of detection (LOD) and quantification (LOQ), intra and inter-day imprecision (n=15), bias (n=15), extraction efficiency (n=6), matrix effect (n=10), process efficiency (n=6), carryover and endogenous and exogenous interferences. As a proof of concept, we analyzed two authentic samples obtained 40 and 240min post-exposure from rats dosed with 2mg/kg MDPV.

Results: The method was linear 5-1,000ng/g with 1/x weighting, except 4-OH-3-MeO-PV that used quadratic model with 1/x weighting. The LOQ was 5ng/g and the LOD was 1ng/g. The intra-day and inter-day imprecision and bias at low QC (15ng/g) and high QC (800ng/g) were within the established criteria (CV<20%, bias 80-120%). Matrix effect was not significant for 4-OH-3-MeO-PV (-10.1%, CV=3%); however, MDPV and 3,4-catechol-PV showed ion suppression (-37.9%, CV=2.2%, and -37.8%, CV=5%, respectively). The extraction efficiency was 91.7% for MDPV, 87.2% for 4-OH-3-MeO-PV, and 38.1% for 3,4-catechol-PV. The process efficiency was 57 % for MDPV, 78.4% for 4-OH-3-MeO-PV, and 23.7% for 3,4-catechol-PV. No carryover was detected after the injection of the upper limit of quantification (1,000ng/g). No interferences were observed. The rat brain collected 40min post-exposure was positive for MDPV (>1,000ng/g), 4-OH-3-MeO-PV (14.9ng/g) and 3,4-catechol-PV (6.3ng/g); 240min post-exposure the sample was positive for MDPV (260.1ng/g) and 4-OH-3-MeO-PV (11.4ng/g).

Conclusion/Discussion: We developed an analytical method to determine MDPV and its metabolites, 4-OH-3-MeO-PV and 3,4-catechol-PV, in rat brain samples with high sensitivity (LOQ 5ng/g in 50mg of sample) and specificity (retention time, 2 MRM transitions, ion ratio). MDPV showed higher concentrations than its metabolites in brain. The method will be applied to additional authentic rat brain samples dosed with different MDPV amounts, to study the pharmacokinetics and pharmacodynamics of MDPV and its metabolites in rats. A better understanding of the pharmacokinetics and pharmacodynamics of MDPV in animals and humans is needed to extrapolate data between species.

POSTER

P75: A Snapshot of Fentanyl Analogs and Designer Opioids: Butler County, Ohio (2016 – 2018)

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Background/Introduction: For the last several years, fentanyl, fentanyl analogs, and other illicit opioids of a nonpharmaceutical origin have been found on the drug market in counterfeit tablets, street 'heroin' products, or marketed on their own as designer substances. These compounds are mu opioid receptor agonists, similar to morphine and oxycodone, and can produce significant adverse effects via central nervous system depression. They have been increasingly cited by forensic pathologists or medical examiners as a cause or contributing cause of death of an individual.

Objectives: The objective of this study was to determine the prevalence of fentanyl analogs and designer opioids in Butler County, Ohio during the years 2016 – 2018. Funding was provided by the Ohio Coroner/Medical Examiner Data Improvement Project (16009-1-1A). A version of this data was presented at the Midwest Association for Toxicology and Therapeutic Drug Monitoring (MATT) annual meeting in April 2019.

Methods: Postmortem whole blood samples were drawn at autopsy from sites such as the femoral and iliac veins and collected in tubes containing sodium fluoride as a preservative. Fentanyl analogs and designer opioids were extracted from postmortem blood samples by two separate methods: a liquid-liquid extraction at alkaline pH into iso-amyl alcohol in n-butyl chloride (for *cis*-3-methylfentanyl, 4-ANPP, acetylfentanyl, acrylfentanyl, betahydroxythiofentanyl, butyrylfentanyl/isobutyrylfentanyl, cyclopropylfentanyl, furanylfentanyl, methoxyacetylfentanyl, ocfentanil, parafluorobutyrylfentanyl/parafluoroisobutyrylfentanyl, tetrahydrofuranfentanyl, and U-47700) and a protein precipitation with cold acetonitrile (for carfentanil). Instrumental analysis was completed by ultra-performance liquid chromatography with triple quadrupole mass spectrometry (LC-MS/MS) operating in positive electrospray ionization mode. All analytical methods were validated according to routine in-house standard operating procedures and protocols based on SWGTOX and SOFT/AAFS guidelines. All cases analyzed in this study were previously tested under the laboratory's comprehensive toxicology scope of analysis.

Results: A total of 467 postmortem blood samples were analyzed for fentanyl analogs and designer opioids during the study. Of those 467 samples, 292 samples (62.5% positive rate) were positive for at least one fentanyl analog or designer opioid. Nine analytes (4-ANPP, acetylfentanyl, acrylfentanyl, butyrylfentanyl, cyclopropylfentanyl, furanylfentanyl, methoxyacetylfentanyl, parafluorobutyrylfentanyl, and U-47700) were detected in the scope of testing while 4 analytes (cis-3-methylfentanyl, beta-hydroxythiofentanil, ocfentanil, and tetrahydrofuranfentanyl) were not detected in any blood specimen. Blood concentrations for all analytes had median values less than 760 pg/mL, with exception of methoxyacetylfentanyl (2,085 pg/mL) and U-47700 (1,155 pg/mL). Of particular note, there were two specific spikes of carfentanil positive casework occurring in June 2016 and February 2017 with subsequent declines to baseline. Age of decedents with positive results for all detections was 18-63 years (mean, 38 years; median, 36 years). 70% of positive casework was male and 30% was female. Other common drugs detected alongside the analytes of interest were fentanyl, benzodiazepines (alprazolam), opiates (6-acetylmorphine, morphine), cannabinoids (THC, THC-COOH), amphetamines (methamphetamine, amphetamine), and ethanol.

Conclusion/Discussion: Fentanyl analogs and designer opioids were prevalent in Butler County, Ohio during 2016-2018. The prevalence data indicates volatile trending which is measured in months and not years. Blood concentrations detected were consistently very low (sub-ng/mL) and recommended limits of detection are 10-50 pg/mL (0.01-0.05 ng/mL).

POSTER

P76: Drug Chemistry Analysis Results Submitted to the National Forensic Laboratory Information System (NFLIS-Drug) in 2018.

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Introduction: The National Forensic Laboratory Information System (NFLIS) is a program of the Drug Enforcement Administration (DEA), Diversion Control Division. NFLIS-Drug systematically collects drug identification results and associated information from drug cases submitted to and analyzed by Federal, State, and local forensic laboratories. These laboratories analyze controlled and noncontrolled substances secured in law enforcement operations across the country. The NFLIS-Drug participation rate, defined as the percentage of the national drug caseload represented by laboratories that have joined NFLIS, is currently more than 98%. NFLIS-Drug includes 50 State systems and 104 local or municipal laboratories/laboratory systems, representing a total of 283 individual laboratories. The NFLIS-Drug data are used to support drug scheduling decisions and to inform drug policy and drug enforcement initiatives nationally and in local communities around the country.

Objectives: The objective of this presentation is to present the results of drug cases *submitted* to State and local laboratories from January 1, 2018, through June 30, 2018, which were *analyzed* by September 30, 2018. The data presented include *all* drugs mentioned in the laboratories' reported drug items.

Methods: National estimates of the top 25 drugs submitted to State and local laboratories are presented. Semiannual national trends for the estimated number of prescription drug reports that were identified as fentanyl, alprazolam, oxycodone, hydrocodone, buprenorphine, and amphetamine are presented. Major drug categories such as synthetic cannabinoids and narcotic analgesics are also presented. Selected drugs or drug categories by regional trends are presented. As available, we will update presented data for drug cases *submitted* to State and local laboratories from January 1, 2018, through December 30, 2018, which were *analyzed* by March 30, 2019.

Results: From January 1, 2018, through June 30, 2018, an estimated 450,392 distinct drug cases were submitted to State and local laboratories in the United States and analyzed by September 30, 2018. From these cases, an estimated 772,078 drug reports were identified. Methamphetamine was the most frequently reported drug (180,549 reports), followed by cannabis/THC (174,226 reports), cocaine (115,425 reports), and heroin (68,376 reports). These four most frequently reported drugs accounted for approximately 70% of all drug reports. Nationally, alprazolam reports showed an overall increase from the second half of 2003 to the first half of 2010, followed by a decrease through 2013 and a significant increase from 2014 to the first half of 2016. Oxycodone reports showed steady increases from 2001 to 2004, dramatic increases from 2006 to 2010, then a steady decline through the first half of 2018. Methamphetamine was the most frequently reported drug in the West (47%) and South (24%) regions, and cannabis/THC was the most frequently reported drug in the Midwest (25%) and Northeast (27%) regions. Fentanyl accounted for 43% of narcotic analgesic reports. Alprazolam accounted for 59% of tranquilizer and depressant reports. Among identified synthetic cannabinoids, 5F-ADB and FUB-AMB accounted for 67% of the reports

Conclusion/Discussion: Attendees will gain an understanding of NFLIS-Drug as a comprehensive information system that includes data from forensic laboratories that handle the Nation's drug analysis cases. NFLIS publicly shares aggregated and analyzed data through various reports throughout the year including national and regional trends of drugs submitted to State and local laboratories, as presented here. These publications can benefit laboratory managers by increasing their awareness of emerging substances and other drug trends.

Keywords: National Forensic Laboratory Information System, Drug Enforcement Administration, Drug Trends

POSTER

P77: Evaluation of Trace Elements Levels in Commercially Available Kratom Powder and Tea in NC using ICP-OES

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Background/Introduction: Kratom (*Mitragyna speciosa*) is a plant originally from Thailand and Southeast Asia whose leaves produce a great variety of alkaloids with opioid-like analgesic-effects. Due to its stimulant and opioid-like effects, the recreational use of kratom is a huge concern in several countries, from the European Union to the U.S. Its popularity relies on the fact that people believe kratom is nonaddictive and can be used as an alternative treatment for opioid withdrawal or chronic pain. In the US, kratom is mainly consumed as a powder or as a hot beverage (kratom tea). Kratom is produced in dried leaf form and can be easily contaminated with heavy metals from the environment during the plant growth and from the powder manufacturing process. Overexposure of trace elements commonly present in herbal preparations can lead to several toxic effects.

Objectives: To evaluate the levels of trace elements in kratom powder and kratom tea commercially available in North Carolina by ICP-OES. The evaluation of trace elements is a key step in the monitoring of toxic levels of essential and nonessential elements for quality control of herbal products.

Methods: Three different strains of Kratom powder (green Vein, Red Vein, and White Vein) were purchased locally. Tea samples were prepared by brewing 2 g of kratom powder in 100 mL of boiling water. Tea samples were filtered and acidified, and powder samples were digested using nitric acid digestion according to the USP standard method. Digested samples were analyzed using a simultaneous Varian 710 ES axial ICP-OES with CCD detector. Levels of Al, As, B, Ba, Ca, Cd, Cr, Cu, Fe, Mg, Mn, Ni, Pb, Se, and Zn were monitored.

Results: The aluminium, arsenic, chromium, copper, manganese, nickel, and zinc average concentrations in tea were between 36.9-153.5 µg/100 mL, 0.2-0.02 µg/100 mL, <0.01 µg/100 mL, 2.8-5.2 µg/100 mL, 1943.5-2869.2 µg/100 mL, 0.5-0.6 µg/100 mL, and 79.9-88.2 µg/100 mL, respectively. For the powder, concentrations were between 210.6-406.9 mg/kg, 0.1-0.03 mg/kg, 1.6-4.0 mg/kg, 12.3-12.8 mg/kg, 1207.2-1738.9 mg/kg, 1.0-1.4 mg/kg, and 27.1-36.1 mg/kg, respectively. Both tea and powder levels of trace elements compare well with those reported for tea samples from Thailand. Arsenic was detected only in the red and white vein strains products and chromium was detected only in the powder, showing the inefficiency of Cr to be leached from the powder to the hot water. Considering the average intake of kratom tea reported by users as 10 cups or 8 g of kratom powder per day, the results showed a toxic daily intake of manganese. Manganese is considered toxic when consumed in amounts higher than 11 mg/day. All three strains showed daily intake levels of manganese between 9.7-13.9 mg/day, and 29.2-32.9 mg/day for the powder and tea, respectively.

Conclusion/Discussion: Three strains of kratom (Red Vein, Green Vein, and White Vein), widely consumed in North Carolina contained both essential and toxic elements in a wide range. The daily intake of most of the elements analyzed was below the standard limits and may not constitute a health risk, with the exception of manganese levels which were higher than the toxic levels. Manganese overexposure can cause clinical symptoms resembling Parkinson's disease, impaired cognitive development, and increased hyperactive behaviors. Manganese is mainly absorbed in the gastrointestinal tract and can accumulate in the liver, brain, and bones. Manganese levels are not currently monitored by quality control agencies such as FDA, and the risk of toxicity effects due to overexposure of manganese in kratom users must be brought to the attention of the scientific community.

Keywords: kratom, manganese, ICP-OES.

POSTER

P78: Drug Screening using the Randox Evidence Analyzer – NPS II Validation in Whole Blood

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Background/Introduction: In forensic toxicology, the success of drug screening methods can be pivotal in the quality of services provided to the customer such as turnaround time and scope of analysis. Periodic review of drug screening methods and instrumentation should be performed in order to keep a laboratory in the best position to address the needs of increasing caseloads and ensure a relevant scope to include testing for novel psychoactive substances (NPS). In 2017, the National Safety Council updated their recommended scope, cutoffs, and drugs included within Tier I and Tier II for DUI testing. Evaluation of compliance with these recommendations promotes greater standardization of drug testing.

Objectives: To validate the Randox NPS II biochip designed for the rapid screening of case samples for novel psychoactive substances.

Methods: Validation of the NPS II biochip in whole blood on the Randox Evidence analyzer included evaluation of the following parameters: precision, sensitivity, specificity, robustness/reproducibility, previously analyzed casework, cross-reactivity, interference, and false positives/false negative rates per SWGTOX guidelines. Cutoffs were selected based on manufacturer's recommendations and biological relevance. The drugs/drug classes screened by this biochip were as follows: furanylfentanyl, acetylfentanyl, carfentanil, sufentanil, ocfentanyl, AH-7921, MT-45, U-47700, W-19, etizolam, clonazepam, mitragynine, naloxone, and buprenorphine/norbuprenorphine.

Results: Precision was assessed at 50% below the cutoff, at the cutoff, 50% above the cutoff, and two times the cutoff. Target CVs ranged from 6-23%. All but two targets had CVs < 20%. The two CVs > 20% were at 50% below the cutoff. Approximately 50 previously analyzed whole blood case samples were screened by the NPS II assay to determine false positive and false negative rates. False negatifve and positive rates were low which indicated the assay had the ability to properly distinguish between a positive and negative sample at the selected cutoffs. As a component of the manufacturer's validation, Randox evaluated specificity, cross reactivity, and limit of detection and issued that information with the NPS kit.

Conclusion/Discussion: It is crucial to routinely evaluate the effectiveness of practices and procedures in order to identify where improvements can be made. Validation of new methods to increase the scope of analysis and evaluation of current methods to gauge productivity and efficiency are all worthwhile focuses to enhance the services of a forensic laboratory. This study used these approaches to improve the services provided to customers and now provides the opportunity and flexibility to screen for NPS such as fentanyl analogues and designer opioids/benzodiazepines by immunoassay if deemed necessary in the future.

POSTER

P79: Characteristics of Opioid Overdose Deaths of Unintentional and Undetermined Intent among Current or Former Military Personnel, 25 U.S. States, July 2016—June 2018

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Background/Introduction: In 2017, drug overdoses resulted in 70,237 deaths in the United States; 47,600 (67.8%) involved an opioid. Active-duty military personnel and veterans are at increased risk of opioid misuse and experience fatal overdoses at nearly twice the rate of the U.S. adult population. Active-duty military personnel and veterans experience increased rates of depression, post-traumatic stress disorder (PTSD), and other mental health disorders compared to non-military personnel. Veterans with mental health disorders are more likely to receive opioid prescriptions and experience more adverse events than veterans without mental health disorder. In addition, substance use disorders are more common in veterans. However, unintentional drug overdose deaths in current or former military personnel has not been widely investigated.

Objectives: We evaluated opioid overdose deaths of unintentional and undetermined intent in current or former military personnel (CFMP) in 25 states during July 2016–June 2018 to determine the most common substances involved in overdose deaths to inform programs for preventing opioid misuse and opioid-related overdose among CFMP.

Methods: CDC's Enhanced State Opioid Overdose Surveillance program funds 32 states and DC to abstract data from death certificates and medical examiner and coroner reports, including toxicology results, on opioid-involved deaths through the State Unintentional Drug Overdose Reporting System (SUDORS). We compared demographics, mental health diagnoses, and substances involved in opioid overdose deaths among CFMP to non-military personnel. Categorical variables were compared using chi-square analyses.

Results: During July 2016–June 2018, data on 39,490 opioid overdose deaths in 25 states were entered into SUDORS; 36,677 (92.9%) of decedents had information on military status and 2,114 (5.8%) were CFMP. Among these, the median age was 50 years, 93.3% were male, and 80.4% were non-Hispanic White. There were statistically significant differences in sex, age, race/ethnicity, marital status, and education when comparing CFMP to non-military personnel. Approximately 17% of CFMP had a documented history of PTSD compared to 2.6% non-military personnel (p<.0001). Almost 22% of the CFMP were in treatment for a mental health or a substance use disorder at the time of death compared to 19% non-military personnel (p=0.0044). Fentanyl was the most common substance contributing to death or detected on toxicology for both CFMP and non-military decedents (68.2% and 70.4%, respectively). The second most common occurring substance contributing to death or detected on toxicology among CFMP and non-military decedents was prescription opioids (63.2% and 62%, respectively). Approximately 86% of all decedents had two or more substances contributing to death or detected on toxicology. Compared to non-military personnel, CFMP decedents were less likely to have benzodiazepines (29.1% vs. 32.5%, p=0.0014) and methamphetamine (11.1% vs. 13.7%, p=0.0006) as co-occurring substances contributing to death or detected on toxicology. However, CFMP were more likely to have alcohol as a co-occurring substance contributing to death or detected on toxicology than non-military personnel (26.4% vs. 21.1%, p<.0001).

Conclusion/Discussion: While fentanyl contributed to a large number of deaths among all decedents, prescription opioids remain an important contributor to overdose deaths, which highlights the need for continued work to improve opioid prescribing. The Departments of Defense and Veterans Affairs have developed clinical practice guidelines for opioid therapy for chronic pain and management of mental health disorders to promote the safe and effective use of opioids as well as improving treatment of mental health disorder among veterans. In light of the frequency of PTSD and other mental health issues among CFMP decedents, findings suggest there is a need to continue to improve access to mental health treatment and identify opportunities for prevention. Lastly, this study also highlights the impact of polysubstance use in opioid overdose deaths overall suggesting the need for multifaceted and comprehensive approach to prevent drug overdose deaths.

POSTER

P80: Yes, Syn Cann!!

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Background/Introduction: With the continuous evolution of illicit drugs in our communities synthetic cannabinoids have remained quite elusive. The sheer number available in combination with the difficulty associated with their detection, has made these illicit drugs a source of frustration for forensic laboratories. Due to their unique chemical characteristics, extracting them and accurately identifying them in biological specimens can be complex. Consequently, many laboratories send select cases, based on history and investigation, to reference laboratories for screening and quantitation if needed.

Objectives: The objective was to develop and validate a method to identify a variety of synthetic cannabinoids in postmortem specimens; and to apply it to medical examiner cases in which synthetic cannabinoid abuse was indicated in the case history, in scene paraphernalia, and/or if use of the substance was witnessed.

Methods: A Thermo Scientific Dionex Ultimate 3000 Ultra High Performance Liquid Chromatograph (UHPLC) coupled to a Bruker AmaZon Speed Ion Trap mass spectrometer (Ion Trap-MS^n) equipped with ToxTyper™ software was validated for the screen of a broad range of synthetic cannabinoids. Method validation was in accordance with the Scientific Working Group for Forensic Toxicology (SWGTOX) guidelines, including assessment of limit of detection, carryover, interferences, and stability. Specimens (blood, serum, or urine) were fortified with a mixture of 4 deuterated internal standards and extracted using Agilent Plexa PCX solid phase extraction (SPE) columns.   UHPLC separation was achieved using a gradient elution on a Thermo Scientific Acclaim RSLC C18 column (100mm x 2.1mm x 2.2μm). The ion trap was operated using electrospray ionization (ESI) negative polarity. Data was collected using an autoMS^n mode, targeting known precursor and product-precursor ions to produce MS^3 spectral detail. Acquisition was achieved using a data-dependent Scheduled Precursor List (SPL) which contains 63 targeted compounds (Table 1). Out of the 63 targeted compounds, 32 (*italicized* in Table 1) were validated according to SWGTOX guidelines.

 Results: Separation of the 32 validated synthetic cannabinoids was achieved within the 11 minute run time. Limits of detections ranged from 0.1- 5 ng/mL. A mixture of 56 common drugs as well as liver and tissue samples were evaluated for potential exogenous interferences. No drug interferences were observed but liver and brain were not suitable for this analysis. Stability studies indicated extracts remained viable for 12 hours.

Table 1. Analytes included in SYNCANNAB Method

5Cl-AKB48	*Diphenidine*	JWH-200-4-hydroxyindole metabolite
5F-AB-PINACA	*FDU-PB-22*	JWH-210
5F-ADB	*FUB-AMB*	JWH-210-N-(4-hydroxypentyl) metabolite
5F-ADB metabolite 7	*FUB-NPB-22*	JWH-250
5F-AMB	JWH-007	JWH-250-N-(4-hydroxypentyl) metabolite
5F-EDMB PINACA	JWH-015	JWH-307
AB-CHMINACA	JWH-018	JWH-387
AB-FUBINACA	JWH-018-N-(4-hydroxypentyl) metabolite	JWH-398
AB-PINACA	JWH-019	JWH-398-N-(5-hydroxypentyl) metabolite
ACHMINACA	JWH-019-(5-hydroxyindol) metabolite	JWH-412
ADB-CHMINACA	JWH-020	*MA-CHMINACA*
ADB-FUBINACA	JWH-072	*MDMB-CHMCZCA*
AKB48	JWH-073	*MDMB-CHMICA*
AM-1220	JWH-073-N-(3-hydroxybutyl) metabolite	*MDMB-CHMINACA*
AM-2201 N-(4-hydoxypentyl) metabolite	JWH-073-N-(3-hydroxybutyl) metabolite-D5	*MDMB-FUBINACA*
AM-2233	JWH-081	*MMB2201*
AM-694	JWH-081-N-(5-hydroxypentyl) metabolite	*MMB-CHMICA*
CUMYL-THPINACA	JWH-122	*MO-CHMINACA*
D4-AB-FUBINACA (ISTD)	JWH-122-5-fluoropentyl-derivate	*PB-22*
D4-MAB-CHMINACA (ISTD)	JWH-122-N-(4-hydroxypentyl) metabolite	*XLR-11*
D9-AB-PINACA (ISTD)	JWH-200	*XLR-11 D5 (ISTD)*

Conclusion/Discussion: Synthetic cannabinoid screening was successfully applied to postmortem cases in which history indicated use, the presence of paraphernalia found at the scene, or witnessed events. To date, the method has successfully identified synthetic cannabinoids in approximately 50 cases from both District 11 and District 14 Medical Examiner Departments. Most common synthetic cannabinoids identified were 5-Fluoro-ADB, 5-Fluoro-ADB Metabolite 7, and MMB2201. To ensure the method is current, synthetic cannabinoids trends are being tracked and analytes added as they emerge in cases and reports.

P81: Synthetic benzodiazepines New to Georgia Part 1: "Car in Ditch" A Review of Cases with Clonazolam, Flualprazolam, or Flubromazolam and Incident Reports

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Background: Synthetic benzodiazepines, like other novel psychoactive substances, vary over time and location. Clonazolam, flual-prazolam and flubromazolam are relatively new to Georgia. In the summer of 2018 clonazolam and flualprazolam were added to the routine LC/MS/MS screen for DUID cases and detected almost immediately. Since that time, the cases containing these three synthetic benzodiazepines have accumulated and have surpassed all other benzodiazepine and "z" drugs except alprazolam and clonazepam.

Objectives: To learn more about the effects of these drugs on drivers and the patterns of use.

Methods: We examined 117 DUID toxicology reports completed by the GBI and reviewed 103 incident reports graciously provided by the submitting agencies from 54 counties in Georgia.

Discussion: We reviewed 93 clonazolam cases, 17 flualprazolam cases, and 8 flubromazolam cases. The first clonazolam and flualprazolam cases were submitted in May of 2018 by metropolitan Atlanta agencies. The first flubromazolam case was submitted a year earlier in June of 2017 in a South Georgia accident case. About half of the cases reviewed were the result of a single or multi-car accident. Poly drug findings are increasingly common in DUID cases, and this was true of synthetic benzodiazepine cases. For clonazolam 45%, flualprazolam and flubromazolam 36% of reports had *more than three* additional drugs. Cannabinoids (THC, 11-OH-THC, THC-COOH) were the most frequently reported other drug class. For clonazolam cases, 25% had THC concentrations >5 ng/mL. Subject admission of "Xanax" was common refrain in incident reports. Especially true for clonazolam cases, 50/51 cases which had at least one other benzodiazepine, had alprazolam. In the few incidents where pills were recovered and subsequently tested, the pills that appeared to be "Xanax" were found to contain clonazolam and alprazolam. Drivers under the influence of clonazolam, flualprazolam or flubromazolam were expected to exhibit manifestations consistent with a benzodiazepine. Central nervous system depression ranging from moderate to severe was feature of nearly every incident report where manifestations were recorded. Similarly, observed driving behavior or nature of the motor vehicle accident were also consistent with depressants. (Failure to maintain lane, sleeping behind the wheel, leaving the road way, failure to observe traffic signals, disorientation, etc.). Only in a handful of these cases might the manifestations observed be attributed primarily to a synthetic benzodiazepine.

A single case had two synthetic benzodiazepines and was typical of many of the other cases:

	Incident Detail
Demographics	32 y/o male
Driving Behavior	At ~4AM the driver exited interstate "collided into a concrete triangle median… a cross walk signal pole, and then continue to travel across the intersection…" [4 lanes] until hitting another concrete median finally coming to a stop.
SFSTs	HGN (0/6 clues) watery, glassy eyes. Dilated pupils. WAT (5/8 clues) lack of balance and coordination, failure to follow instructions OLS (3/4 clues) same as WAT
Other observations	Lack of convergence, body sway, eyelid tremors, odor of marijuana. Pills & smoking devices were recovered.
Subject admission	Hydrocodone, Xanax, Suboxone, occasional marijuana use
Toxicology (blood)	THC 6.1ng/mL, 11-OH-THC 2.0 ng/mL, THC-COOH 34 ng/mL, tramadol 63 µg/L, hydrocodone less than 12.5 µg/L alprazolam 25 µg/L, flualprazolam, and clonazolam

Conclusions: Clonazolam, flualprazolam, and flubromazolam reports in DUID cases rose alarmingly in the last year. Alone or in combination with other drugs, they can impair psychomotor function. Toxicologist vigilance for the emergence of new psychoactive substances in their area and complimentary screening techniques is essential.

POSTER

P82: Synthetic Benzodiazepines New to Georgia Part 2: Analysis and Short Term Stability Study

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Introduction: The Toxicology Section for GBI-DOFS Crime Laboratory has seen an increasing number of illicit and newer prescription benzodiazepines. In the course of analysis, it was observed that cases found to contain clonazolam in the initial LC/MS/MS screen, could no longer be positively identified later during confirmation testing. Stability of these drugs is of considerable interest due frequent delays between drug screening and final drug confirmation caused by growing caseloads.

Objectives: This study evaluated the short-term stability of 11 newer benzodiazepines in human whole blood preserved with NaF/$K_2C_2O_4$, when stored at refrigerated temperatures (~4°C). The cross-reactivity by immunoassay of several of these benzodiazepines was also evaluated.

Method: Gray-stoppered blood collection tubes, containing 2mL blood, were spiked to concentrations of 100 micrograms/liter of clobazam, clonazolam, diclazepam, flualprazolam, flubromazepam, flubromazolam, flunitrazolam, loprazolam, lormetazepam, meclonazepam and zolazepam. A 200 mcL aliquot was removed from each tube and extracted via protein precipitation with acetone, followed by filtration and LC/MS/MS analysis using Agilent ZORBAX Eclipse Plus C18 Column. Positive mode electrospray ionization MS analysis was performed using multiple reaction monitoring (MRM) mode (see below for transitions). The instrumentation used for analysis was either the Applied Biosystems, Inc. QTRAP 3200 with attached Agilent 1260 Liquid Chromatography system or Applied Biosystems, Inc. QTRAP 4500 with attached Shimadzu Exion LC Liquid Chromatography system. Diazepam-d5 and clonazepam-d4 were used as internal standards (ISTD) and were added to each aliquot prior to analysis. Samples were analyzed in triplicate every week for the first 6 weeks, and will continue on a monthly basis for 4 months. The cross-reactivity of clonazolam and flualprazolam were analyzed using CEDIA® benzodiazepine immunoassay with a 200 ng/mL cut-off and a Beckman Coulter AU480 analyzer.

Drug	Class	MRM transition	Limit of Identification LOD/LOI	Quantitation ISTD
Clonazepam-d4	ISTD	320/274		
Diazepam-d5	ISTD	290/198		
Mepivacaine	RRT ISTD	248/298		
Clobazam	Prescription	301/259	3.125 µg/L	*Diazepam-d5*
Clonazolam	NPS	354/308	3.125 µg/L	*Clonazepam-d4*
Diclazepam	NPS	321/154	3.125 µg/L	*Diazepam-d5*
Flualprazolam	NPS	327/223	3.125 µg/L	*Diazepam-d5*
Flubromazepam	NPS	333/226	12.5 µg/L	Diazepam-d5
Flubromazolam	NPS	373/ 223	12.5 µg/L	*Clonazepam-d4*
Flunitrazolam	NPS	338/ 292	3.125 µg/L	*Clonazepam-d4*
Loprazolam	prescription	465/111	12.5 µg/L	*Diazepam-d5*
Lormetazepam	prescription	337/ 291	3.125 µg/L	*Diazepam-d5*
Meclonazepam	NPS	330/284	3.125 µg/L	*Clonazepam-d4*
Zolazepam	veterinary	287/ 138	3.125 µg/L	*Clonazepam-d4*

Results: Analyte concentrations were determined by a 7-point calibration curve, ranging from 12.5 to 200 micrograms/liter. All analytes were initially quantitated off all three internal standards in order to determine the best fit for purpose. All calibrations had a R value of >/= 0.980 and %CV of less than 20%. The limit of detection (LOD/LOI) was determined for all analytes. Most of the analytes appear to be stable at refrigerated temperatures for the 6-week study period, with the exception of clonazolam and loprazolam, which decreased by ~20% and 40%, respectively. The immunoassay cross-reactivity for clonazolam was approximately 70% and flualprazolam was approximately 124%. In cases reviewed, about 20% of clonazolam only cases would have been considered indicatvely positive benzodiazepines. For flualprazolam only cases, about 50% were indicative.

Conclusion: The short term stability of seven NPS benzodiazepine and three prescription benzodiazepines were studied. Clonazolam and loprazolam concentrations decreased over the 6-week study period. Rapid confirmation after first detection of these compounds, especially at concentrations near the limit of detection, should be accomplished whenever possible.

P83: Evaluation of the Abuse Potential of Methcathinone Using Intracranial Self-Stimulation in Rats

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Background/Introduction: Intracranial self-stimulation (ICSS) is a behavioral procedure that has been used primarily in rats to evaluate abuse potential of drugs. ICSS involves surgical implantation of an electrode into the brain's medial forebrain bundle, a component of the dopaminergic reward pathway implicated in the physiology of addiction. Subjects can activate the electrode by pressing a response lever, and this contingent electrical stimulus reinforces further lever-pressing behavior. The administration of compounds with high abuse potential generally increases ICSS response rates to a degree that correlates with other preclinical measures of abuse potential (e.g. drug self-administration). We use this ICSS procedure to evaluate methcathinone, the β-ketone analog of methamphetamine. Methcathinone is a well-characterized molecule of the synthetic cathinone class and will be used as a comparison other drugs within this class. Our results suggest that ICSS has utility in predicting which emerging Novel Psychoactive Substances (NPS) are likely to have high potential for abuse by individuals and for proliferation within a community.

Objectives: The objective is to evaluate ICSS as a model for determining abuse potential of NPS, using methcathinone as an example.

Methods: Subjects were adult male Sprague-Dawley rats (n=6) with surgically implanted electrodes targeting the medial forebrain bundle. Rats were placed in operant-conditioning chambers equipped with a response lever and ICSS stimulator, and each lever press resulted in delivery of a contingent 0.5-sec train of 100-μsec square-wave pulses. Behavioral sessions consisted of sequential 10-min components, and during each component, the frequency of pulses declined from 158 Hz to 56 Hz in 0.05 logarithmic increments, with each frequency available for a 1-min frequency trial. During training, the amplitude of electrical current was modified until each subject responded at high rates for the highest four to five frequencies and at low rates or not at all at the lower frequencies. Test sessions consisted of three consecutive baseline components followed first by intraperitoneal injection of methcathinone (0, 0.10, 0.32, and 1.0 mg/kg) and then by two test components. The number of stimulations earned during each trial was expressed as percentage of the Maximum Control Rate (%MCR; MCR defined as the mean of the maximum number of stimulations earned during any trial of each baseline component). To provide a summary measure of data collapsed across all frequencies, the number of stimulations earned during each component was expressed as a percentage of the baseline number of stimulations per component. %MCR and % Baseline Stimulations were averaged across test components within each rat and then across rats for a given methcathinone dose. %MCR data were analyzed by a two-way repeated measures ANOVA with dose and frequency as factors. % Baseline Stimulations data were analyzed by a one-way repeated measures ANOVA with dose as the single factor. A significant ANOVA was followed by a Holm-Sidak post-hoc test ($p<0.05$).

Results: Methcathinone produced a dose-dependent increase in ICSS expressed as leftward shifts in ICSS frequency-rate curves. Analysis of frequency-rate curves indicated that both 0.32 and 1.0 mg/kg methcathinone significantly increased ICSS rates relative to vehicle treatment across a range of frequencies. Similarly, analysis of the total number of stimulations per component indicated that both 0.32 and 1.0 mg/kg methcathinone significantly increased % Baseline Stimulations. The 0.1 mg/kg dose did not significantly affect ICSS rates relative to vehicle.

Conclusion/Discussion: Methcathinone-induced ICSS facilitation is consistent with methcathinone's high abuse potential. Despite its high abuse potential, methcathinone's lack of wide proliferation as an agent of abuse indicates that this is not the only factor influencing propagation. ICSS can be used to assess the abuse potential of NPSs and guide allocation of limited resources of a forensic toxicology laboratory in responding to emerging threats.

POSTER

P84: Evaluation of the Long-Term Stability of Select Phenylacetylindole, Cycloalkyolindole, Quinolinyl, and Carboxamide Synthetic Cannabinoids in Human Whole Blood Using LC-MS/MS

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Background/Introduction: Despite efforts to control synthetic cannabinoids, clandestine manufacturers continue to modify their structures to avoid legal consequences, creating an ever-changing analytical target for forensic laboratories. Forensic toxicology laboratories often lack the needed resources or do not have the capabilities to test for these compounds and metabolites, requiring specimens to be submitted to reference laboratories. Drug stability can be affected by long storage times, temperature and preservatives. Although these factors can be controlled, systematic research is necessary to identify their impacts on the stability of these new synthetic cannabinoids that are continually emerging.

Objective: The purpose of this research is to assess the stability of 17 synthetic cannabinoids in human whole blood using liquid chromatography-tandem mass spectrometry (LC-MS/MS) over twenty-one weeks. The method was validated in accordance to the Academy Standards Board method validation guidelines for quantitative analysis and stability evaluation of the following analytes: phenylacetylindoles JWH-250 and RCS-8; cycloalkylindoles UR144 and XLR11; quinolinyls PB-22 and NM2201; and carboxamides 4-cyano CUMYL-BUTINACA, 5-fluoro-3,5-ABPFUPPYCA, 5-fluoro ADB-PINACA, 5-fluoro PY-PINACA, ADB-PINACA, APP-PICA, CUMYL-THPINACA, EMB-FUBINACA, MDMB-FUBICA, MEP-CHMICA, and MO-CHMINACA.

Methods: Stability under room temperature, refrigerator temperature (2-8 ºC) and freezer temperature (-20 ºC) at high (10 ng/mL) and low (1.5 ng/mL) concentrations each in triplicate were evaluated at the select nine time points: 0 hour, 24 hours, 72 hours, 1 week, 3 weeks, 5 weeks, 9 weeks, 17 weeks, and 21 weeks. Blood was preserved with sodium fluoride prior to the stability study. Extraction of analytes was conducted using supported liquid extraction (SLE+) ISOLUTE cartridges (Biotage, Charlotte, NC, USA). The extracts were analyzed using a Waters XBridge reverse-phase C18 column (3.5 µM, 2.1 x 50 mm, Milford, MA, USA) by Shimadzu HPLC with a SCIEX 4000 Q-Trap Electrospray Ionization Tandem Mass Spectrometry (ESI+/MS/MS) in positive ionization mode. The total run time was 8 minutes with a 0.6 mL/min flow rate and 10 µL injection volume.

Results: Linear calibration curves for each analyte had acceptable R^2 values > 0.99 using a weighting factor of 1/x. A linear dynamic range of 0.1 – 25 ng/mL was used for all analytes within acceptable ± 20% calculated bias and imprecision. No signs of carryover were observed. Analytes were considered stable if the average area ratio at the time point was within ± 20% of the average area ratio response at time point zero. Phenylacetylindoles and cycloalkylindoles that were stable up to 21 weeks under all temperatures, such as JWH-250, RCS-8 and UR144, generally had a core structure of a carbonyl substituent on a pyrrole with surrounding nonpolar groups of hydrocarbons and heterocyclic rings; whereas compounds with two polar carbonyl functional groups present, such as EMB-FUBINACA, 5-fluoro ADB-PINACA, and APP-PICA, were found to experience degradation a lot earlier at 1 week or less in room temperature and refrigerator storage conditions. 5-fluoropentyl analogs, like XLR11 and 5-fluoro ADB-PINACA, in comparison to their counterpart analyte, UR144 and ADB-PINACA, were unstable at earlier time points of less than 1 week under room temperature and/or refrigeration.

Conclusion: The validated method demonstrates a sensitive and reliable way to positively identify 17 different synthetic cannabinoids in human whole blood in rapid time for stability analysis. Further, the use of SLE improved sample preparation efficiency by decreasing the extraction time from 1 hour to 30 minutes compared to traditional extraction methods, such as solid-phase extraction and liquid-liquid extraction. The select 17 synthetic cannabinoids generally degraded in the order of room temperature, refrigerator, then freezer temperature. Long-term stability results revealed that the overwhelming majority of synthetic cannabinoids were stable up to 21 weeks when kept frozen.

Keywords: Synthetic Cannabinoids, Supported Liquid Extraction, Stability

POSTER

P85: The Evolution of Novel Psychoactive Substances (NPS) Detected in Toxicological Casework

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Background/Introduction: The novel psychoactive substance (NPS) phenomena is a global issue that has resulted in hundreds of new substances of all different classes to flood the recreational drug market. One of the biggest challenges involving NPS is identifying popular substances and subsequently developing testing capabilities to confirm these drugs in biological specimens. Drug supply and availability, geographical and population influences, and drug control actions such as legislation ultimately affect the life span of any one substance, which is typically transient in nature. This translates to rapid changes in drug positivity, which may outpace availability of toxicological testing. Understanding which group of NPS affects the largest number of cases helps toxicology laboratories to prioritize those analytes for test development and validation.

Objectives: To highlight the breadth of substances in various NPS subclasses detected in blood submitted in toxicological casework, with emphasis on specific substances and subclasses with the highest positivity.

Methods: Toxicological data from blood specimens obtained during the course of death investigation, impaired human performance, and suspected overdose cases were evaluated for the presence of NPS submitted between 2014-2018. During that period, more than 14,200 NPS results were reported in over 11,000 blood samples.

Results: Between 2014-2018, more than 120 different NPS have been encountered, with the majority being classified as synthetic cannabinoids. More than 45 different synthetic cannabinoids are represented in approximately 2500 blood results, with the most prevalent compound shifting from JWH-018 to XLR-11 to AB-PINACA to 5F-ADB over time. Ten cathinones, including methylone, *alpha*-PVP, and *N*-ethylpentylone have accounted for the majority of over 1050 blood positives. Following the emergence of acetylfentanyl, another 20 different novel illicit opioids have been reported in casework. Designer opioids are the most prolific group of substances, with over 8100 blood confirmations with this classification, with the top five compounds including furanylfentanyl, para-fluoroisobutyrylfentanyl (FIBF), carfentanil, U-47700 and cyclopropylfentanyl. Designer benzodiazepines have been confirmed in over 800 cases, with etizolam as the most frequent finding, but this group also includes phenazepam, flubromazolam, flubromazepam, diclazepam and delorazepam. Mitragynine, the primary plant alkaloid found in *Kratom* products which are being used in Western societies typically as a natural alternative for pain management, is being encountered more frequently in casework, with confirmation in more than a 1000 samples to date. Designer dissociatives like methoxetamine and PCP analogs, as well as designer hallucinogens, like the NBOMe group, were found in smaller numbers (<100 total).

Conclusion/Discussion: The sheer number of NPS, representing an increasing number of chemical classes, along with the many shifts in NPS popularity over time has inevitably resulted in a growing list of emerging substances. This growing list of NPS poses a quandary to toxicology laboratories as they attempt to identify the compounds which to apply their limited resources, specifically in regards to reference material procurement and development and validation of screening and confirmatory methodologies for a variety of biological specimens. Prioritization of emerging substances should be based on evaluation of current drug trends, regional tendencies, and provenance of submitted casework. For example, designer opioids are a frequent finding in postmortem casework, while the detection of designer benzodiazepines may have a greater impact on driving under the influence investigations. The drug monitoring community must continue to evaluate the shifting trends and ensure that testing capabilities are subsequently updated and used to screen all samples to truly capture the true scope of NPS.

P86: Detection and Quantitation of Cannabidiol and delta(9)-Tetrahydrocannabinol in Oral Fluid of a Therapeutic-Use Cannabidiol Donor Using UHPLC-Laminar Flow-MS/MS

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Background/Introduction: Cannabidiol (CBD) is one of over 80 active cannabinoids found in *Cannabis Sativa* and is the second most abundant cannabinoid derived from the plant following Δ(9)-Tetrahydrocannabinol (THC). As opposed to THC, CBD does not appear to have any psychotropic effects. Legislation regarding the therapeutic or recreational use of CBD varies, however many individuals use CBD products for management of seizures, anxiety, insomnia, and more.

Objectives: During the extraction of CBD from plant material, THC may be co-extracted. Therefore, screening and quantitating potential THC levels in individuals using CBD products is important in instances where the legality of use of THC does not match that of CBD.

Methods: This project evaluated the detection and quantitation of CBD, THC, and two primary metabolites in oral fluid samples of a therapeutic-use cannabidiol donor using Supported Liquid Extraction (SLE) and subsequent testing by PerkinElmer QSight® 220 CR LC-MS/MS (PerkinElmer, Waltham, MA) in positive ionization mode using a PerkinElmer® Brownlee C18 2.1x50mm (2.7 μm) column. A method was developed for the detection and quantitation of THC, CBD, 11-hydroxy-Δ(9)-THC, 11-nor-9-carboxy-Δ(9)-THC, Δ(9)-THC-d3, and 11-hydroxy-Δ(9)-THC d3 (Cayman Chemical, Ann Arbor, MI, USA).

All samples, calibrators, and quality controls were prepared by spiking certified reference standards into synthetic oral fluid (UTAK, Valencia, CA, USA). Calibrators were prepared at 1, 2.5, 5, 10, 20, 30, and 50 ng/mL, to evaluate the calibration model and to identify the limit of quantitation (LOQ) and limit of detection (LOD) with quality controls analyzed at 3, 15, and 40 ng/mL. Samples were prepared in 5% glacial acetic acid (Acros, New Jersey, USA). SLE was performed using ISOLUTE SLE+ 1mL columns (Biotage AB, Uppsala, Sweden) with elution in hexane:ethyl acetate:MBTE (80:10:10), followed by evaporation. All samples were reconstituted in 100μL of 0.1% formic acid (Sigma-Aldrich, St. Louis, MO, USA) in DI water:0.1% formic acid in acetonitrile (70:30). Validation parameters were assessed using ASB Standard 036-Standard Practices for Method Validation in Forensic Toxicology, including carry over, LOD, LOQ, linear dynamic range, internal standards interferences, and calibration model.

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Results: All compounds were quantified using linear calibration models. The linear dynamic range was determined to be 1 to 50 ng/mL with a LOQ of 1 ng/mL and a LOD of 0.5 ng/mL. Carryover was assessed by running a double blank following a sample spiked at 50 ng/mL with no analytes observed. Oral fluid samples spiked with only deuterated internal standards were used to determine any potential interferences and none were observed. Total run time including equilibration was eleven minutes.

The donor samples were collected at several timepoints during the oral administration of an 8mg dose of CBD. These timepoints included prior to administration, at the time of administration, 30 minutes post administration, 45 minutes post administration, 60 minutes post administration, 90 minutes post administration, and 120 minutes post administration. CBD was quantified within the samples from below LOD to >50 ng/mL, above the highest calibrator. THC was quantified within the samples from below LOD to 0.7 ng/mL. Metabolites were not detected above the LOD.

Conclusion/Discussion: Overall, the use of laminar flow mass spectrometry was effective in detecting various cannabinoids in oral fluid samples following SLE sample extraction.

P87: Forensic Screening and Quantitation of Various Novel Psychoactive Substances (NPS) using a High-Resolution Accurate Mass System

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Background/Introduction: Synthetic cannabinoids and cathinones make up the majority of NPS commonly encountered. Analyzing these compounds is challenging: limited information is available on these drugs and the chemical composition and potencies are highly variable. Therefore, these substances continue to pose serious public health and safety issues. With a large number of deaths caused by these NPS each year, timely and comprehensive drug screening approaches are needed in the forensic laboratory to rapidly and accurately identify these emerging novel substances.

Objectives: In this study, we aimed to evaluate analytical performance of a screening method for the accurate quantification of a panel of 54 NPSs including synthetic cathinones, synthetic cannabinoids, benzodiazepines, and fentanyl analogs using the SCIEX X500R QTOF system. We compared two different sample preparation techniques: (1) protein precipitation and (2) salt assisted liquid-liquid extraction (SALLE).

Methods: Protein Crash: Human whole blood was fortified at calibration concentrations ranging from 0.1 to 200 ng/mL for all analytes. For the protein precipitation samples, 100 μL of K2 EDTA blood was spiked with various levels of analytes and internal standards. Four hundred μL of cold acetonitrile was added to the mixture and the sample was vigorously vortexed for 10 seconds, and centrifuged at 15,000 g for 5 min. 360 μL of the supernatant were transferred to a clean 2-mL Eppendorf tube and the sample dried down under nitrogen gas. The sample was reconstituted in 144 μL of 80:20 Water: MeOH.

SALLE: 100 μL of K2-EDTA blood was mixed with IS and 300 μL of saturated KCl solution. The sample was vortexed and 3 mL of cold acetonitrile was added. The solution was left to settle and 2 mL from the organic layer were transferred to a clean glass tube, dried, and reconstituted with 120 μL of 80:20 Water: MeOH.

Chromatography: Compounds were chromatographically separated using a Water/Methanol gradient with ammonium formate and a Phenomenex 50 x 4.6 mm Kinetex® 2.6 μm Phenyl-Hexyl column. The total LC runtime was 9.5 min.

Mass Spectrometry parameters: A X500R QTOF system was used in the analysis of all samples, and the MS data collected using SWATH with variable windows.

Data was acquired and processed with SCIEX OS 1.5.

Results: Both protein precipitation and SALLE performed adequately in terms of extraction efficiency for all the analytes that were evaluated. LC separation allowed baseline separation of majority of the analytes in the panel. A separate and longer LC method was tested for fentanyl analogs to achieve better separation due to the presence of several isobaric groups. All of the 54 analytes were evaluated with the limit of detection (LOD) between 0.1 to 0.2 ng/mL in human whole blood. The R^2 values of the calibrations curves of these analytes were all >0.99.

Conclusion/Discussion: In this study, we evaluated the analytical performance of a screening method for the accurate quantification of 54 NPS in forensic whole blood samples using the SCIEX X500R QTOF system. Two sample preparation techniques were evaluated and compared: protein precipitation and SALLE. Both approaches yielded similar recovery.

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P88: Advancing Forensic DUID Screening with Mass Spectrometry

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Background/Introduction: Quadrupole Time-of-flight mass spectrometry (QTOF-MS) provides high-resolution, accurate-mass data for full-scan information of both precursor ion and all product ions. This is an ideal approach for forensic toxicology screening where unknown compounds in complex biological samples must be identified from information-rich data sets. In this technical note, a comprehensive drug screening workflow for the analysis of forensic Driving Under the Influence of Drugs (DUID) blood samples is described.

Objectives: The objective of this study is to use this novel DUID method to obtain retention times and MS/MS spectra necessary to build a targeted analysis workflow for 60 compounds. The adaptation of QTOF-MS technology should enable identification of multiple number of the targeted compounds present in authentic DUID case samples in comparison to immunoassay-based screening.

Methods: Control whole blood samples were fortified with a stock standard solution mixture and extracted for LC-MS screening to determine retention times. Forensic DUID case samples and control whole blood samples were extracted by using a protein precipitation prior to being reconstituted in mobile phase for analysis.

Analytes were chromatographically separated at 30˚C using a Phenomenex Kinetex phenyl-hexyl (50 x 2.1 mm, 2.6 μm) column. Mobile phase was ammonium acetate in water and ammonium acetate in acetonitrile plus formic acid, 500 μL/min flow rate. Mass spectrometric detection was conducted on a X500R QTOF System operating in positive electrospray mode with information dependent acquisition (IDA) MS/MS method and negative electrospray mode with multiple reaction monitoring (MRM) MS/MS method, respectively. Two different acquisition strategies were employed to streamline the screening workflow. Samples were evaluated against four main confidence criteria weighted as follows: mass error (15%), retention time (30%), isotope ratio difference (5%), and library score (50%) for all compounds. These criteria were used to generate a combined score. The processing criteria for positive identification of an analyte in a sample required all four main confidence criteria to pass.

A total of 30 DUID case samples were evaluated during the course of this study. Comparison between immunoassay-based detection and QTOF-MS-based detection was made in terms of sample volume required for analysis, number of kits or assays needed per sample and levels of sensitivity (LOD) and selectivity (false positive rate).

Results: The average (n=9) combined scores obtained for all 60 target analytes in control blood samples spiked at the LOD were found to be above 80% (except THC-COOH with a combined score of 70.1%). It was found that THC-COOH had sufficient S/N ratios (> 200) and mass error less than 1 ppm at the LOD (10 ng/mL) for positive identification. However, low-abundance MS/MS spectra were obtained at that concentration level, subsequently resulting in an average combined score of 70%. Further optimization on the sample extraction protocol is recommended to enhance THC-COOH sensitivity and MS/MS fragmentation. Inter-day reproducibility resulted in %RSDs ranging between 1-10% for the target analytes. DUID case samples were examined with both immunoassay analyzer and the SCIEX X500R QTOF System. For the majority of the case samples, target analytes were specifically detected using QTOF-MS but missed or classified as a single compound class (e.g., *OPI* for Opiates and metabolites) by the immunoassay approach.

Conclusion/Discussion: The implementation of a robust method development process resulted in high combined scores for all compounds based on the four main confidence criteria defined in the processing method. Positive ionizable compounds were acquired using the IDA-MS/MS acquisition mode, as it enabled the easy collection of precursor ions, and multiple dependent MS/MS scans on several of the most abundant precursor/candidate ions. For the remaining target compounds that favor negative electrospray ionization, MRM was used as the acquisition strategy as it was shown to provide additional selectivity monitoring unique parent ions.

Additional quantification strategies were implemented by setting an analyte concentration threshold based on the LODs to minimize false positives and/or false negative hits, which resulted in LODs in the sub ng/mL range, mass errors less than 2 ppm and MS/MS scores over 90%. Average combined scores based on multiple acceptance criteria (Retention Time, Mass error, Isotope ratio, MS/MS library hit and concentration) ranged between 70-98% for all target analytes, resulting in successful compound identification. Overall, the developed QTOF-MS screening approach was compared to the traditional immunoassay-based screening method. The adaptation of QTOF-MS technology enabled the use of microliter volumes of blood samples (as opposed to milliliter volumes for immunoassay-based detection), while meeting NSC-ADID cutoff recommendations. This benefit eliminated the use of multiple immunoassay reagent kits used for screening. In addition, the developed QTOF-MS screening approach enabled the identification of multiple number of the targeted compounds present in authentic DUID case samples in comparison to immunoassay-based screening, showing the robustness and reproducibility of the overall workflow.

P89: Ultra-Sensitive Forensic Analysis of Cocaine and its Metabolites in Spiked Hair Samples

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Background/Introduction: Cocaine is one of the most commonly abused recreational drugs, with an estimated 16.5 million people or 0.35% of the worldwide population abusing the substance.[1] Detection of its use can be performed in several biological matrices such as blood, urine, oral fluid and hair. While urine and oral fluid are very useful for determining cocaine use in short term, hair samples are becoming extremely valuable in testing the long-term use. In this technical note, a sensitive and reliable analytical workflow is presented which combines the use of mass spectrometry (MS) and Solid Phase Extraction (SPE).

Objectives: The objective of this study is to determine a method for quantification of cocaine and its metabolites in hair. This should be accomplished by the development of a robust and optimized extraction procedure to maximize analyte recovery in combination of a high sensitivity LCMS method. Using this strategy, sub pg/mg lower limits of quantification (LLOQ) in hair matrix is achieved for cocaine metabolites.

Methods: The panel of analytes used in this study included cocaine and ten of its metabolites (ecgonine, ecgonine methyl ester, benzoylecgonine, norcocaine, p-OH-benzoylecgonine, m-OH-benzoylecgonine, cocaethylene, m-OH-cocaine, o-OH-cocaine and p-OH-cocaine. Cocaine-d3 was used as the internal standard. Hair samples were washed accorded to accepted laboratory proce-dure, dried out, cut into segments and incubated overnight at 45˚C for complete digestion. The digested hair samples were cooled to room temperature prior to being spiked with a 1 μg/mL stock standard solution mixture and extracted for LC/MS screening using a solid phase extraction procedure. Prior to analysis, the hair samples were reconstituted in mobile phase for analysis.

Analytes were chromatographically separated at 30˚C using a Phenomenex Kinetex Biphenyl column (100 x 3 mm, 2.6 μm) column. Mobile phases were formic avid in water and methanol, 600 μL/min flow rate. Mass spectrometric detection was conducted on a QTRAP® 6500+ mass spectrometer operated in positive electrospray mode with multiple reaction monitoring (MRM) MS/MS meth-od.

Results: The recovery and matrix effect were calculated using 0.005 ng/mg of each internal standard to assess the recoveries of the analytes following the SPE procedure. Analyte extraction recoveries were demonstrated to be greater than 80%, enabling the analytical workflow to obtain sub pg/mg lower limits of quantification (LLOQ) in hair matrix for the two hydrococaine isomers. The calculated values showed that the extraction procedures yielded excellent recoveries of the analytes of interest.

Following the SPE procedure, 10 μL of the reconstituted solution were injected for each compound. Calibration curves were gen-erated for each of the compounds to determine limits of quantitation (LOQ). The results demonstrated excellent linearity of the generated regression curves covering linear dynamic range from 3 to 4 orders of magnitude (1 ng/mg to 0.1 pg/mg); coefficients of variations (CVs) within 10% and good accuracies for all target analytes. Signal-to-noise ratios (S/N) at LLOQ were found to vary from 10 to 50. The workflow showed excellent accuracy (>95%) and precision (<15%), with excellent linearity resulting in R^2 values of 0.9990 for all analytes. Table 1 summarizes the lower limits of quantitation (LLOQ) for the target analytes used in this study.

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Table 1. Lower Limits of Quantitation (LLOQ) for Cocaine and Metabolites Panel.	
Analyte	LLOQ (ng/mg)
Ecgonine	0.05
Ecgonine Methyl Ester	0.0025
Benzoylecgonine	0.001
Norcocaine	0.0005
Cocaine	0.0005
p-OH-Benzoylecgonine	0.01
m-OH-Benzoylecgonine	0.01
Cocaethylene	0.0001
m-OH-Cocaine	0.00005
o-OH-Cocaine	0.00005
p-OH-Cocaine	0.001

Conclusion/Discussion: Using this method, the QTRAP MS was used to acquire full scan MS/MS spectra containing the complete molecular fingerprint of cocaine and its metabolites. These MS/MS spectra can be searched against relevant library for confirmation of detection. Using this strategy, simultaneous identification and confirmation of cocaine and its metabolites by acquiring full MS/MS data and using automated MS/MS library searching.

Reference:

1.   Peacock A, Leung J, Larney S, Colledge S, Hickman M, Rehm J, et al. Global statistics on alcohol, tobacco and illicit drug use: 2017 status report. Addiction. 2018. DOI: 10.1111/ add.14234.

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P90: Quantitative Analysis of Fentanyl Analogues in Human Whole Blood Utilizing Improved Throughput with Multiplexing Technology

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Background/Introduction: Fentanyl analogues and their metabolites are a rising concern as thousands die from opioid overdose across the country. Some of these synthetic drugs have very high potency and thus only require a small amount for an accidental overdose. In addition, these high potency drugs pose a danger to public health and public safety personnel due to the possibility of skin absorption or inhalation of the drug. In order to properly identify these fentanyl analogues in biological matrices, forensic laboratories require sensitive MS systems to accurately quantitate at low concentrations, and highly specific chromatographic methods in order to separate and properly identify isomers.

Objectives: A confirmatory method for 29 fentanyl and fentanyl analogues in human blood was developed. Due to the inclusion of isomers with similar fragmentation patterns, the separation of these isomers within the panel was key in method development, in order to accurately identify and quantitate all fentanyl analogues.

Methods:

Preparation: A calibration curve was prepared in methanol and spiked into human whole blood to give desired concentrations ranging from 0.1 ng/ml to 100 ng/ml in order to evaluate the dynamic range.

1. 40 µL of corresponding methanolic standard was spiked into 360µL of human whole blood. 20 µL of internal standard spiking solution was added, along with 1.14 mL of cold acetonitrile.

2. Samples were vortexed vigorously and centrifuged.

3. 500 µL of supernatant was transferred, dried down and reconstituted with 125µL diluent solution (mobile phase).

4. Subsequent sample was centrifuged, and supernatant was transferred to LC vial for analysis.

Liquid Chromatography: HPLC separation was achieved using a Phenomenex C18 column at 30°C on the SCIEX ExionLC™ AC system. Mobile phase consisted of water, methanol, acetonitrile and modifiers. The LC flow rate was 0.3 mL/min and the total run time was 17 min. The injection volume was 5 µL. Utilization of Sciex Multiplexing (MPX) would reduce total run time.

Mass Spectrometry: Data was collected using positive electrospray ionization on the QTRAP® 4500 system with Analyst 1.7 software.

Validation Protocol: Several key criteria were evaluated during the validation process. These criteria included HPLC separation resolution, LOQ, carryover, precision and linear dynamic range. These factors were evaluated in human blood matrices.

Results: Nine fentanyl analogues were isomeric with other analogues on panel requiring chromatographic separation from their isomers for confident identification and quantitation. Separation was accomplished by using a Phenomenex C18 column which allowed for better retention and selectivity of the more polar analytes throughout the gradient. The column in conjunction with an optimized mobile phase composition produced the separation that was needed to correctly distinguish all isomers (excluding p-FBF/m-FBF and p-FIBF/m-FIBF). Analogues p-FBF and m-FBF, as well as p-FIBF and m-FIBF, were unable to be separated chromatographically so they were combined to be analyzed together as p-FBF/m-FBF and p-FIBF/m-FIBF. Another improvement is the reduction of the HPLC runtime from 17 minutes to 11.5 utilizing MPX.

Conclusion/Discussion: The QTRAP 4500 system combined with the ExionLC AC system enabled the separation of fentanyl, its analogues, and metabolites, while maintaining the sensitivity at low concentrations in complex biological matrices such as human whole blood.

• Baseline separation of fentanyl analogues and isomers

• LC chromatographic method can be used on other platforms, including the SCIEX X500R QTOF, for screening and confirmatory techniques.

• Dynamic range for quantitation averaged ~4 orders of magnitude across the compounds monitored in this study.

• By utilizing Sciex MPX total run time can be reduced to 11.5 minutes.

P91: Generation of Presumptive Metabolites of a Novel Synthetic Opioid Using Human Liver Microsomes and Subsequent Analysis by Orbitrap LC-MS/MS

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Background/Introduction: The synthetic opioid 2-methyl AP-237 was originally synthesized and reported in Japan in the 1970s. Related analogs have been used internationally for pain management in the clinical setting but never in the US. This compound has recently appeared for sale on the dark web and there has been discussion of its use on Reddit and drug user forums. This substance is therefore of potential concern to forensic chemists and toxicologists around the world.

Objectives: To identify the potential sites of phase I metabolism of 2-methyl AP-237 and propose the structures of the main metabolites.

Methods: Metabolites of 2-methyl AP-237 were produced using *in-vitro* metabolism conducted by incubation with 10-pool human liver microsomes (HLM) and analyzed using orbitrap LC-MS/MS. In addition, the structures of the metabolites of 2-methyl AP-237 were predicted computationally using Schrodinger's quantitative structure activity relationship (QSAR) model based on intrinsic reactivity in the presence of CYP3A4. To rank the hydrophobic footprint of 2-methyl AP-237 metabolites, Desmond molecular dynamics were used to estimate the probability of intra-molecular hydrogen-bonding.

Results: The HLM incubation with 2-methyl AP-237 yielded four main mono-hydroxylated metabolites (303.21 m/z). One of the metabolites (M1), was able to be assigned by the presence of the fragments at 133.06 m/z and 171.15 m/z, indicating hydroxylation at the *meta* or *para* position of the phenyl ring. Metabolites M2, M3, and M4 (Figure) all contained the fragment 117.07 m/z, indicating that hydroxylation had taken place elsewhere on the molecule. Based on the intrinsic CYP450 reactivity QSAR model, terminal hydroxylation of the propyl amide (M2) and hydroxylation of the beta position of propyl amide (M3) were assigned to be among the four most probable metabolites. The primary metabolite (by abundance), had a longer retention time (5.62 min) than the parent compound (5.42 min) and metabolites M1 (4.67 min), M2 (4.80 min), and M3 (5.22 min). A Molecular dynamics simulation was used to assign M4 (methyl group hydroxylation) as the primary metabolite because it was found to have the highest probability of intra-molecular hydrogen bonding among all four metabolites.
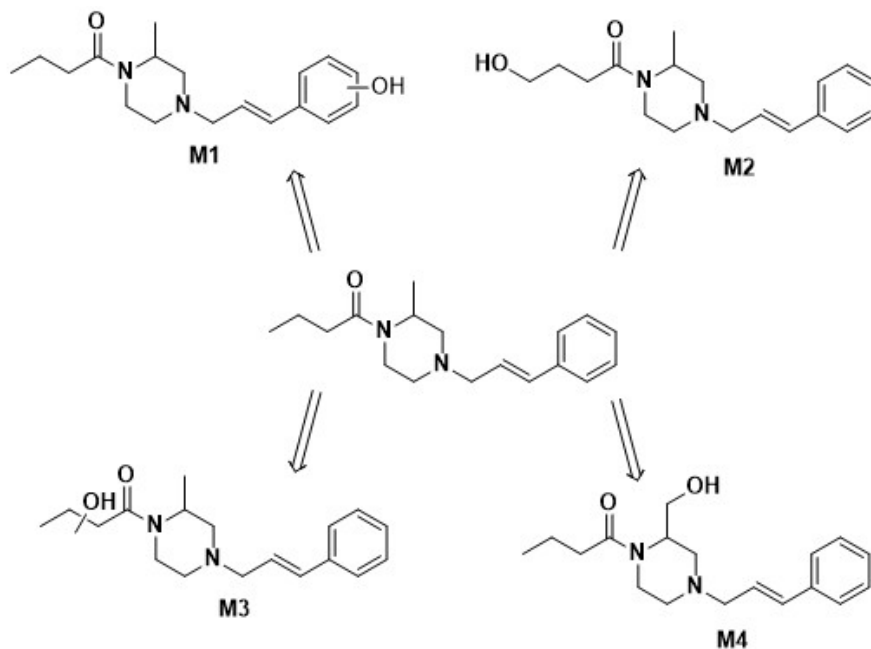

Figure 1: Probable metabolites of 2-methyl AP-237 identified by LC-MS/MS fragmentation

Figure 1: Probable metabolites of 2-methyl AP-237 identified by LC-MS/MS fragmentation

Conclusion/Discussion: The microsomal assay of 2-methyl AP-237 produced four main phase I metabolites (M1, M2, M3, M4), of which hydroxylation of the methyl group on the piperazine ring appears to be the primary metabolite. The Schrodinger CYP intrinsic reactivity QSAR model and Desmond molecular dynamics can be used to help predict phase I metabolites of novel psychoactive substances.

P92: First Identification of Adrafinil and Modafinil, Its Metabolite, in Human Hair. About a Controlled Study to Interpret an Authentic Case

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Background/Introduction: The toxicology laboratory of the Institute of legal medicine of Strasbourg test in routine hair specimens for clinical, forensic and doping cases. Recently, the laboratory was requested to test for adrafinil in hair. A 34-year-old escort girl was arrested for adrafinil trafficking. The police found more than 100 small vials of adrafinil in her belongings. She declared that it was for her personal consumption, to have more courage for her difficult job. She denies being a trafficker. A urine test was realized and revealed the presence of modafinil, the main metabolite of adrafinil, but no trace of the parent molecule. At the same time, a hair specimen (12 cm, brown) was collected. Adrafinil was discovered in the late 1970s, by Lafon Laboratories, to treat neurological symptoms of elderly subjects. However, the drug is often diverted and used for recreational use, or sports doping. Adrafinil is classified as a prohibited substance by World Anti-Doping Agency and accounted for 0.2 % of the substances identified in 2017 (1 case out of 4076). There is little literature about cases where adrafinil was identified in blood or urine. Nothing is published about analysis of adrafinil or modafinil in human hair. The target compound (adrafinil, modafinil, etc.) and the expected concentrations are unknown.

Objectives: In order to be able to interpret results from the real case, a mini-controlled study was realized with oral administration to a volunteer and beard collection 10 days after administration.

Methods: Adrafinil powder was bought on Internet and analyzed by nuclear magnetic resonance (NMR) spectroscopy (Avance 400, Bruker Biospin) to confirm its nature and its purity. No organic impurity was detected in the powder and purity was established at 94.6 %. A healthy volunteer was administered a 200 mg oral dose of adrafinil. The therapeutic dosage is between 300 and 1200 mg per day. His beard hair (0.4 cm) was collected 10 days after the exposure. A specific method to test for adrafinil and modafinil was developed on an ultra-high performance liquid chromatography (Acquity class I) coupled to a Xevo TQD tandem mass spectrometer (UHPLC-MS/MS) from Waters, using liquid-liquid incubation/extraction at pH 4.0 and specific transitions m/z 312.0 > 145.0 and 312.0 > 152.0 for adrafinil, and 296.0 > 129.0 and 296.0 > 152.0 for modafinil. The limit of quantification was fixed at 0.1 ng/mg.

Results: After a single exposure of adrafinil, the volunteer's beard hair test result was positive for adrafinil (0.8 ng/mg) and for modafinil (0.5 ng/mg).

The hair strand, from the trafficking case, was segmented (4 x 3 cm) to establish the historic pattern of adrafinil use. The results are presented in Table 1.

Table 1: Results of the hair strand.

Segments (cm)	0-3	3-6	6-9	9-12
Adrafinil (ng/mg)	ND	ND	ND	ND
Modafinil (ng/mg)	13.9	13.2	12.3	6.8

No adrafinil was identified, but modafinil tested positive at 6.8 to 13.9 ng/mg. The toxicological significance of the measured concentrations is difficult to determine because this is the first case describing hair analysis for modafinil.

Conclusion/Discussion: According to the results of the controlled study, after a single exposure of adrafinil, both adrafinil and modafinil are detectable in hair. The results obtained for the analysis of the hair strand from the trafficking case do not match with a consumption of adrafinil, as no adrafinil was identify. In this way, it appears that the young woman was not consumer of adrafinil, but of modafinil, a well-known stimulant. Although not studied nor reported, one can anticipate good adrafinil stability in hair, as it is the case for all other drugs when the specimen is stored dry at room temperature.

P93: Characterization of Nicotine, Glycols, and Ethanol in New Zealand E-liquids: Evidence of Ongoing Global Quality Control Issues

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Background/Introduction: Electronic cigarettes (e-cigs) are an alternative to smoking traditional cigarettes and are viewed as smoking cessation products. Traditional e-cigs have an e-liquid containing nicotine, propylene glycol (PG), vegetable glycerin (VG), and flavorants. Laws regulating e-cigs vary from country to country but in New Zealand, e-cigs and e-liquids fell under the Medicines Act of 1981 and the Smoke Free Environment's Act of 1990 which prohibited their sale and distribution. This ban was lifted in May of 2018, but sales are still restricted to those under the age of 18.

Objectives: The objective of this study was to characterize e-liquids purchased in New Zealand during the legal transitory period of e-liquids. The objective was to quantify nicotine, glycols, ethanol and identify flavorants present in the New Zealand e-liquids. Aerosol particle size of a standard 10% ethanol 50:50 PG:VG e-liquid was evaluated.

Methods: Twenty-two e-liquids were purchased from multiple vape shops throughout New Zealand with labeled concentrations of nicotine from 0 to 50 mg/mL. E-liquid samples were analyzed in triplicate   at dilutions of 1:10,000 or 1:100,000. Nicotine-d$_4$ was added as the internal standard. Quantitation was performed on an Applied Biosystems 6500 QTrap with a Turbolon Spray attached to a Shimadzu UHPLC-MS/MS system. Chromatographic separation was performed using Zorbax Eclipse XDB-C18 column (3.5 μm x 4.6 x 75 mm) at an isocratic flow rate of 0.5 mL/min with 10 μL injection. For glycol quantitation, e-liquids were derivatized with benzoyl chloride and analyzed on a Waters AcQuity Xevo-TQ-S Micro LC-MS/MS using a BEH C18 column (1.7 μm x 2.1 x 100 mm). For ethanol analysis, e-liquids were diluted 1:10 and 20 mg/L t-butanol was added as the internal standard. Quantitation was performed on a Shimadzu GC 2030 with HS-20 headspace. Particle size was evaluated using a 10 stage Micro-Orifice Uniform Deposit Impactor (MOUDI) and ethanol was quantitated from each stage. A 10% ethanol by mass 50:50 PG:VG e-liquid was aerosolized with a KangerTech e-cig (n=10) for 10 seconds with a vacuum of 30 L/min. Ethanol was analyzed by Shimadzu 2014 GC-FID with a Tekmar HT3 headspace. The flavorants were screened using a DART JEOL JMS T100 LC ACCU-TOF™ with a scan range from 40 to 1000 *m/z*.

Results: Measured nicotine concentrations were within ±10% in 10 of the 22 e-liquids (45%). The remaining e-liquids ranged from -88% to +12% of the labeled concentrations. Of the nine samples with PG and/or VG information, 4 were labeled as "Max VG" with measured PG:VG ratios of 20:80. One was labeled as "VG" but was 32:68 PG:VG. Of the remaining four samples labeled, one labeled as "≥55% PG and ≤25% VG" was determined to contain 31:69 PG:VG. The final 3 samples were reasonably close to the label contents. Twelve samples (55%) which did not list ethanol as an ingredient contained ethanol 0.03% to 2.50% ethanol (*w/v*). The mean mass aerodynamic diameter for an e-liquid prepared with 10% ethanol and 50:50 PG:VG was 0.38 ± 0.08 μm. Compounds typically used for flavoring were identified.

Conclusion/Discussion: The results of this study show that legislation for quality assurance and quality control needs to be enacted to protect the public. Measured nicotine, PG, and VG concentrations varied from labeled concentrations of the randomly purchased products. Ethanol was not listed as an ingredient in any products. Clarity is needed on what ingredients are present and at what concentration.   More research needs to be conducted to evaluate the impact of inhaled ethanol from e-cigarettes.

POSTER

P94: Melanin Content in Hair: Validation and Application to DFSA Cases

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Background/Introduction: The analysis of hair for drugs may be used in drug facilitated sexual assault (DFSA) cases to increase the drug detection window. Previous research has demonstrated that the incorporation of many drugs into hair is directly impacted by the melanin content of the hair (1, 2). These drugs include those potentially implicated in DFSA cases, such as morphine and codeine. Postoperative patients are being investigated as an appropriate surrogate for DFSA victims in types of drugs administered and acute vs. chronic dosing.

Objectives: This project developed and validated a method for quantitative determination of melanin content in segmented human head hair that is amenable to large-batch processing and analysis. This method is being used as part of a larger study to provide more detailed information about the detection of drugs such as benzodiazepines, anesthetics, and pain relievers in postoperative patients subjected to a single dose and was tested on a large population of real-life samples.

Methods: The method of Kronstrand et al. (1) was modified to allow for large-batch analysis of samples and validated in accordance with SWGTOX guidelines. Calibration curve and quality control (QC) standards were prepared in melanin-free (albino) human hair spiked with melanin. Hair was decontaminated and cryogenically ground into a fine powder. Hair powder was weighed to ±0.01 mg, with 4 mg used for very dark hair and 8 mg used for very light hair, as determined visually by the analyst. A value between 4 mg and 8 mg was used for intermediate shades of hair. The hair powder was then solubilized in a strong organic base (Soluene 350®, PerkinElmer) in a 2-mL microcentrifuge tube and vortexed for 10 seconds. After digestion at 60 °C for 45 minutes, with vortexing for 10 seconds after 30 minutes, and cooling to room temperature, 250 μL of each resultant solution was pipetted into a 96-well quartz microplate. Absorbance was measured at 500 nm using a Molecular Devices VersaMax™ Microplate Reader.

Results: The goal of validation was to meet SWGTOX guidelines and achieve linearity over the range 10 to 200 μg, with R^2 greater than 0.99. These goals were met, with no weighting. The calibration model was evaluated according to the method of Almeida et al. (3) in which the residuals were plotted against concentration and an F-test performed to test the homoscedasticity assumption. Weighting factors of 1 (no weight), 1/x, and $1/x^2$ based on the percent relative error were evaluated. The method was determined to have an LOD of 3 μg, based on 3.3 times the standard deviation of the y-intercept divided by the average slope. LOQ was determined at 10 μg, based on the lowest non-zero calibrator. Accuracy and precision were within ±8% at 3 levels (10, 100, and 200 μg), which exceeded the goal of within ±20%. The commonly encountered interferent of hair dye was examined by (a) dying albino hair to a very dark shade and (b) bleaching a sample of very dark hair and comparing each to its undyed counterpart with commercial over-the-counter hair dye. As expected, hair dye was found to interfere with the determination of melanin content. Stability was assessed at 1, 2, and 3 days, and after storage in the refrigerator (4 °C). The stability was determined to be acceptable, with all absorbance within ±20% of the average day 0 value, and with less than ±20% bias for all samples (±25% for low standard).

Conclusion/Discussion: The method was fully validated to SWGTOX guidelines and used for multiple batches of more than 70 samples each. This method may be applied to the hair of suspected DFSA victims to provide better interpretation of drug concentrations found in segmented hair.

Using the validated method, samples from a previous large study will be analyzed and the initial results will be presented at the meeting. This pool of subjects includes males and females, as well as Asians, Caucasians, and people of African descent. Melanin content will be analyzed to determine the range of melanin concentrations observed, as well as any differences between genders or races. Hair from graying subjects ("salt-and-pepper") will be separated and gray and dark/colored portions analyzed separately. Melanin content in hair collected at different times from the same individual, along the length of a single sample, and at the same time from different spots on a subject's head will also be determined and reported.

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P95: The Detection of 6-Monoacetylmorphine in ChildGuard®-Tested Hair Specimens Following Reformulation of Oxycodone Tablets

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Background/Introduction: Prescription opioids are often abused through alternative routes of administration, such as injection or nasal insufflation of the drug. In August 2010, Purdue Pharma, the producers of the prescription opioid OxyContin®, replaced their oxycodone formula with an abuse-deterrent formula that made it more difficult to crush and dissolve the pill. Following the reformulation of OxyContin®, there was a 36% decrease in the number of oxycodone exposures classified as an abuse, and a 42% increase for heroin in the National Poison Data System.

Children who are exposed to illicit substances often face other difficulties such as neglect, abuse, and violence. The purpose of ChildGuard® hair testing is to determine if the donor has been exposed to any illegal substance. A positive result in a ChildGuard® hair test suggests that the individual was exposed to drug smoke, to the drug itself, to body fluid from a drug user, or accidentally, or intentionally ingested the drug. The ChildGuard® hair test can detect drug exposure in the individual's environment up to 3 months prior to collection and can be performed regardless of the age of the individual.

Objective: The purpose of this study was to determine if there was a change in the detection of 6-monoacetylmorphine, a heroin metabolite, but also a product of natural heroin decomposition, and oxycodone in ChildGuard®-tested hair specimens, from both children and adults, after the reformulation of oxycodone tablets in 2010.

Methods: The hair specimens tested by ChildGuard® protocol were not washed prior to analysis. All 27,210 hair specimens were screened and confirmed by previously validated enzyme-linked immunosorbent assay and liquid chromatography tandem mass spectrometry, respectively. Positivity rates were determined by dividing the total number of ChildGuard® hair specimens positive for 6-monoacetylmorphine by the total number of ChildGuard® hair specimens analyzed for each 6-month period from 2008 to 2016. A Mann-Whitney test was employed to determine if there was a significant difference in positivity rate and 6-monoacetylmorphine concentrations before and after the reformulation of OxyContin®. The same statistical analyses were performed for oxycodone.

Results: The positivity rate of 6-monoacetylmorphine in ChildGuard® hair specimens was constant from 2008 to 2011 (Mean=0.14% SD=0.001) and from 2012 to 2016 (Mean=0.55%, SD=0.003). There was a statistically significant increase in positivity rates between these two time periods (Mann-Whitney U Value=0, P<0.05). The average concentration of 6-monoacetylmorphine before 2012 (M_1=618 pg/mg; IQR: 254, 2178 pg/mg), and after 2012 (M_2=585 pg/mg; IQR: 289, 1358 pg/mg), has remained consistent. Oxycodone positivity rates in ChildGuard® hair specimens have been slowly increasing since 2008 and did not show any significant change before and after 2012. However, the annual median concentration of oxycodone decreased after 2010 (Mann-Whitney U Value=0, P<0.05, M_1=768 pg/mg, M_2=373 pg/mg).

Conclusion/Discussion: There has been a statistically significant increase in 6-monoacetylmorphine positivity rate in ChildGuard® hair specimens suggesting, as previous literature stated, that the abuse-deterrent OxyContin® formula contributed to an increase in heroin use. The literature reported an increase in early 2011 following the reformulation of oxycodone. Our observed increase occurred in January of 2012 which could be the result of studies being conducted in two different populations and using totally different study designs. In addition, a large supply of old oxycodone formulation could still had been available for abuse. There has also been a steady increase in oxycodone positivity rates since 2008. A limitation of this study was a small sample population – a total of 250 hair samples were positive for 6-monoacetylmorphine between 2008 and 2016.

Keywords: Opiate, 6-monoacetylmorphine, Hair, ChildGuard®

POSTER

P96: Drug Confirmation Analysis in Oral Fluid Using an Automated In-Tip Extraction Technique and LC/MS/MS.

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Background/Introduction: There has been a significant increased interest in oral fluid drug testing within the forensic toxicology field. Oral fluid drug testing is deemed to be desirable due to its non-invasive specimen collection technique and its ability to detect parent drug and reflects recent use. Alabama Department of Forensic Sciences (ADFS) was the first state crime laboratory to offer in-house confirmatory oral fluid drug testing. In-tip Dispersive Pipette eXtraction (DPX) is a non-traditional SPE technique in which loose sorbent mixes with sample solution by simply aspirating and dispensing within a pipette tip. In ADFS, a semi-automated drug confirmation analysis in oral fluid was validated by using DPX with Integra Viaflo 96 and analyzed by LC/MS/MS (QQQ). This method can be transitioned onto a fully-automated pipette system - Hamilton Microlab® STARlet. The scope of testing and cutoffs were determined based on the recommendations established by the National Safety Council's Alcohol, Drugs and Impairment Division (NSC-ADID).

Objectives: To validate an automated in-tip oral fluid extraction method for 20 drugs of abuse in oral fluid.

Methods: Extraction was performed using an Integra semi-automated pipetting unit with DPX mixed mode tips (RP/WAX) that were purchased from DPX Labs, LLC (Columbia, SC). Samples were analyzed by an Agilent 1290 LC (Agilent Poroshell EC-C18 2.1 X 100mm, 2.7 μm column) coupled with a 6460 Triple Quadrupole Mass Spectrometer. The following qualitative validation parameters were evaluated per SWGTOX guidelines: limits of detection, matrix and analytes interferences, robustness, and carryover. Simulated casework was analyzed to assess fit for purpose.

Results: The validated limit of detection was determined to be 0.5 ng/mL for 6-monoacetylmorphine (6-MAM); 1.0 ng/mL for Alprazolam, Amphetamine, Benzoylecgonine, Carisoprodol, Cocaine, Fentanyl, Diazepam, Hydrocodone, Meprobamate, Methadone, Methamphetamine, Morphine, Nordiazepam, Oxycodone, and Zolpidem; 2.0 ng/mL for Buprenorphine; 4.0 ng/mL for Clonazepam and Lorazepam; 10 ng/mL for THC. No interferences from matrix effects or common drugs of abuse were detected. Carryover was evaluated at 100 ng/mL and 400 ng/mL, no carryover was indicated for all targets except cocaine. However, the responses were less than 10% of the limit of detection and were deemed as noise.

Conclusion/Discussion: A qualitative method for drug confirmation in oral fluid was successfully developed and validated using DPX with Integra Viaflo 96 and LC/MS/MS. This method displayed excellent sensitivity, reproducibility, and demonstrated no interference from blank matrices or commonly encountered analytes. In addition, this method allows for quick addition of new targets by conducting sensitivity and supplemental interferences studies per SWGTOX validation guidelines. Furthermore, this semi-automated method can be transitioned to the Hamilton Microlab® STARlet liquid handler by conducting a performance verification. This fully-automated pipetting system uses air displacement pipetting and CO-RE (Compressed O-Ring Expansion) techniques to achieve high accuracy and provides faster analysis process due to its ability to analyze larger sample size (e.g. 96 samples) in each run. Due to new applications of drug testing, toxicology laboratories will benefit from enhancing their methodology to include oral fluid drug testing and incorporating methods that are easily automated.

P97: Detection of Fentanyl in the Oral Fluid of Infants

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Background/Introduction: Fentanyl is a widely used opioid in Neonatal Intensive Care Units (NICU) for its analgesic, sedative, and anesthetic properties in pre-term and term infants. Fentanyl is administered for pain reduction during surgeries, mechanical ventilation, incubation, and/or post-operative relief.

Due to the lack of literature for drug testing of oral fluid in neonates and infants, the ability to detect, as well as, the extent of which metabolism and/or oral fluid composition may affect the detection of opioid drugs in oral fluid is unknown. The presented case studies demonstrate proof of concept that oral fluid in neonates can be a viable specimen for collection and analysis. A non-invasive and quick collection of specimens for the analysis of drugs is critical in improving treatment regimens that better support the health and development of the infant born drug addicted and reduce NICU health care costs can be developed.

Objectives: The objective of this study was to determine the presence of fentanyl in oral fluid of infants administered fentanyl while hospitalized in the NICU at the Children's Hospital of Richmond of Virginia Commonwealth University (VCU). This preliminary study will assess the ability to detect high potency, low dose drugs in neonatal oral fluid that could be beneficial in diagnosing opioid exposure and risk for opioid withdrawal in neonatal abstinence syndrome (NAS) prior to the onset of symptoms.

Methods: Oral fluid collection was approved by the VCU Institutional Review Board. Samples were collected from two infants by rolling a cotton swab along the infant's mouth, inner cheeks, and tongue until saturated. Infant 1, born at 24 weeks gestation with a birth weight of 510 g, received a continuous fentanyl infusion of 7 µg/kg/hr, the sample was collected on the seventh day of treatment, day 147 of life. Fentanyl infusion was increased to 40 µg/kg/hr, the sample was collected on the second day of treatment, day 169 of life. Infant 2, born at 24 weeks gestation with a birth weight of 750 g, received a continuous fentanyl infusion of 4 µg/kg/hr for 11 hours, the sample was collected 5 hours following the termination of treatment on day 89 of life. Swabs containing an estimated volume of 100 µL oral fluid were placed in 2.0 mL of phosphate-buffered saline, centrifuged, and analyzed by ultra-high pressure liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) for fentanyl and norfentanyl after solid phase extraction (SPE) with SPEC MP3 columns (Agilent, Santa Clara, CA).

Results: Infant 1 at a dose of 7 µg/kg/hr had no detectable fentanyl or norfentanyl, however, at a dose of 40 µg/kg/hr contained fentanyl and norfentanyl at estimated concentrations of 28 and 78 ng/mL, respectively. Infant 2 at a dose of 4 µg/kg/hr had no detectable fentanyl or norfentanyl.

Conclusion/Discussion: Limitations of the current study include the stability, recovery, and matrix effects from infant oral fluid collected using a cotton swab. Traditional oral fluid collection devices are not viable options due to their large size and potential chemicals on the pad, which are concerns in the care of neonates. Therefore, a critical evaluation needs to include the impact of using a plain cotton swab for the collection of oral fluid.

The absence of fentanyl and norfentanyl in infant 1 (7 µg/kg/hr) and infant 2 may be attributed to the limited sample size, infant's ability to metabolize fentanyl, and/or current limit of detection, 0.5 ng/mL. The ability to detect fentanyl and norfentanyl in infant oral fluid was demonstrated by infant 1 (40 µg/kg/hr). This study demonstrated the ability to detect high potency, low dose drugs in infant oral fluid.

POSTER

P98: The Detection of Fentanyl in Human Umbilical Cord Tissue: Method Validation and Prevalence in a High-Risk Population

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Background/Introduction: Maternal opioid use continues to be a serious public health issue. In the United States between 1999 and 2014, maternal opioid misuse has risen from 1.5 per 1000 deliveries to 6.5 per 1000 deliveries (p <0.05; MMWR, 2018). According to a recent CDC report, over half of all opioid deaths involve fentanyl (MMWR, 2017), yet fentanyl is not included in most routine newborn toxicology tests (Nellhaus et al, 2018; Colby et al, 2018). Umbilical cord is a truly universal specimen type that is readily available using an easy one step collection procedure and has been gaining popularity as a specimen type for newborn toxicology.

Objectives: The specific aim of this study was to develop and validate a forensically defensible procedure (the use of two different analytical methodologies to derive a result) to detect the presence of fentanyl in human umbilical cord tissue.

Methods: The assay was validated using the guidelines of the Scientific Working Group for Forensic Toxicology (SWGTOX) and the method was used to survey samples of authentic umbilical cord specimens received at a national reference laboratory for routine toxicological analysis. All specimens were subjected to fully validated immunoassay initial test (Fentanyl Direct ELISA Kit) followed by confirmation of presumptive positive specimens using liquid chromatography tandem mass spectrometry (LC-MS/MS) following solid phase extraction (CSDAU, 200 mg bed/10mL cartridge).

Results: The method satisfied all of the criteria recommended by SWGTOX. Following the SWGTOX guidelines for qualitative assays the following were assessed; precision, accuracy, limit of detection, carryover, stability, interference and matrix effect. The %CV's of the precision and accuracy experiments for the immunoassay procedure were within 10% using a cutoff of 500 pg/g fentanyl. The precision and accuracy calculations for the confirmation assay at 200, 500 and 1000 pg/g were within 15% of target values and achieved a limit of detection of 100 pg/g.

Between October 01, 2018 and November 30, 2018, our laboratory received 484 umbilical cord specimens for fentanyl analysis. The immunoassay procedure identified 5 specimens with fentanyl at or above our established 500 pg/g cutoff with controls at 250 pg/g and 1000 pg/g. The five presumptive positive samples were extracted and tested using a qualitative LC-MS/MS procedure with the calibrator set at the cutoff value of 500 pg/g and controls at 200 pg/g and 1000 pg/g. All five specimens contained detectable amounts of fentanyl above the cutoff using our validated LC-MS/MS confirmation procedure.

Conclusion/Discussion: We have presented a fully validated method for the detection of fentanyl in human umbilical cord tissue following the recommendations of the SWGTOX guidelines using an operational screening cutoff of 500 pg/g cutoff. This method will be useful for future epidemiological studies as well as a useful tool for the identification of fentanyl exposed newborns for cases referred for routine toxicological analysis. As the prevalence of illicit fentanyl use continues to grow, the detection of fentanyl in newborns may assist perinatal professionals to properly identify fentanyl exposed newborns.

P99: Analysis of Fentanyl in Authentic Hair Samples

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Background/Introduction: Fentanyl is a synthetic opioid prescribed for pain treatment that also has a high potential for misuse and abuse, making it a pressing public health issue. In the United States for the year 2017 alone, synthetic opioids other than methadone, including fentanyl, accounted for 28,466 drug overdose deaths.[1] In this work, we have developed an enzyme immunoassay (EIA) for the detection of fentanyl in hair. Since hair analysis measures habitual or chronic ingestion over an extended time period, our data may provide insight into fentanyl hair concentrations from ingestion over a broad time frame.

Objectives: The aim of this study is to document our findings of hair samples positive for the synthetic opioid fentanyl using hair samples analyzed by the Psychemedics laboratory.

Methods: Samples (n = 197) from a time period of November 2016 to June 2017 were screened by (1) drug extraction from hair using a patented method (US Patent 8,084,215)[2] and (2) performing an in-house developed microplate immunoassay using a selected monoclonal antibody with a fentanyl cutoff at 20 pg/mg hair and controls at -50/+100% of the cutoff. The immunoassay is designed for qualitative use and has been cleared by FDA (k182103). The confirmation process consisted of a new hair aliquot that was first washed using an extended aqueous method followed by solid phase extraction and quantitation using an AB SciEx 6500 for LC/MS/MS confirmation in positive multiple reaction mode. Cutoff for the confirmation was set at 20 pg fentanyl/mg hair. The LC/MS/MS method was linear from 1 to 750 pg/mg with LLOQ of 1 pg/mg hair for all analytes.

Results: Cross reactivity of the antibody using fentanyl calibrator was butyryl fentanyl (200%), valeryl fentanyl (100%), furanyl fentanyl (100%), acetyl fentanyl (125%), ortho-fluorofentanyl (100%), acryl fentanyl (100%), cyclopropyl fentanyl (100%), isobutyryl fentanyl (100%), octfentanil (100%), 4-fluoro-isobutyryl fentanyl (100%) and norfentanyl (< 0.2%). The effects of cosmetic treatments (permanent wave, dye and bleach, relaxer and shampoo) using fentanyl negative and positive samples in the immunoassay were determined. In this study, all negative hair samples remained negative after treatment, with all positive samples remaining positive after treatment. A comparison study (n = 197) of the immunoassay with LC/MS/MS results after an extended aqueous wash was carried out with stored samples previously received for workplace drug testing by the Psychemedics laboratory. All samples identified as negative by the immunoassay were confirmed as negative by LC/MS/MS (n = 100). Eighty-seven samples identified as positive by the immunoassay were confirmed positive by LC/MS/MS. Ten samples identified as positive by the immunoassay were confirmed below the cutoff but above LLOQ by LC/MS/MS after an extended aqueous wash.

Conclusion/Discussion: This work provides data from an enzyme immunoassay developed to detect fentanyl in human hair samples. To our knowledge, this is the first example of an EIA that has been cleared for use in hair by the FDA.

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POSTER

P100: Systematic Comparison of Decontamination Parameters Associated with the Toxicological Analysis of Human Head Hair

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Background/Introduction: Forensic toxicological analysis for the purposes of protecting human health and supporting criminal justice activities can be achieved, in part, by means of analysis of human head hair for detection of abused substances. As a complex solid sample matrix, hair requires pre-treatment measures including **_decontamination_**, homogenization, and extraction to remove drug from the hair components to allow for analysis. Optimizing pre-treatment parameters is essential for accurate toxicological analysis of this matrix. One of the most challenging issues in hair analysis is differentiating between drug detected as a result of superficial deposition onto hair and drug incorporated from actual use. This interpretive challenge necessitates removal of drug deposited on hair by contamination from an individual's surroundings prior to analysis. Over time, a variety of decontamination methods have been published without systematic comparison.

Objectives: The major goal of this work was to conduct a comprehensive comparison of the efficacy of decontamination variables by means of statistical design of experiments (DoE). DoE designs systematically vary all of the independent variables at once, as opposed to traditional experiments where each independent variable is changed one at a time. This allows for the correlation of variation detected in the dependent variable (_i.e.,_ wash recovery) to individual independent variables (_i.e.,_ different decontamination parameters) and combinations of those independent variables. DoE studies were completed for several drugs with different physicochemical properties (_e.g.,_ hydrophobicity, pK_a, and total polar surface area), such that a comparison could be made between optimal decontamination conditions for different compounds to facilitate greater standardization of the process of hair testing.

Methods: A 2^4 fractional factorial block design, followed by analysis of variance (ANOVA) and Tukey's honestly significant difference (HSD), were used to compare the main factors for the process of decontamination: wash time, aqueous and organic decontamination solvent identities, and order of wash (_i.e.,_ organic wash first vs. aqueous wash first). The blocking factors were the number of sequential washes with both the aqueous and organic solvents. The design matrix was completed in triplicate on hair samples that were intentionally contaminated with a single compound. Quantitation of the drug detected in wash solutions and extracts from hair was accomplished via LC-QqQ analysis.

Results: Statistical analysis of the quantities of drug recovered in the decontamination washes indicated that there were both similarities and differences between optimal decontamination conditions for drugs with different physicochemical properties. In general, multiple washes, with at least one aqueous wash using 1% sodium dodecyl sulfate (SDS), and at least one organic wash was necessary for the adequate decontamination of drug from the surface of hair. For example, it was found that the samples with the highest removal of amphetamine from the surface of hair were decontaminated by washing with three volumes of 1% SDS, then one volume of methanol, for 30 s each wash. Samples contaminated with diazepam and washed three times with 1% SDS before being washed three times with methanol (each wash for 30 min), resulted in the greatest removal of drug from the surface of hair. Samples contaminated with Δ^9-THC were most effectively decontaminated with 30 s washes, first with the organic solvent, followed by one wash with 1% SDS.

Conclusion/Discussion: DoE was found to be a valuable approach to optimizing hair decontamination parameters for different drugs. Optimal parameters for decontamination differ based on the physicochemical and/or structural characteristics of the specific target drug.

POSTER

P101: The Detection and Quantitation of Synthetic Cannabinoids in Hair by LC-MS/MS

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Background/Introduction: Synthetic cannabinoid use is an ongoing public health threat. Detection of these drugs in hair provides an opportunity to identify drug users and monitor drug usage trends in different populations. Hair testing for synthetic cannabinoids represents unique challenges and opportunities due to the patterns of parent drug and metabolite incorporation into hair.

Objectives: We developed a quantitative LC-MS/MS screen for 15 synthetic cannabinoid compounds (JWH-018, JWH-073, AM2201, UR-144, XLR-11, AB-PINACA, 5-fluoro AB-PINACA, AB-FUBINACA, ADBICA, ADB-PINACA, AB-CHMINACA, APINACA, PB-22, 5-fluoro PB-22 and BB-22), and a quantitative LC-MS/MS confirmation for synthetic cannabinoid metabolites (JWH-018 N-(5-hydroxypentyl), JWH-073 N-(4-hydroxybutyl), JWH-073 N-(3-hydroxybutyl), AM2201 N-(4-hydroxypentyl), AB-PINACA N-(5-hydroxypentyl), APINACA N-(5-hydroxypentyl), ADBICA N-(5-hydroxypentyl), ADB-PINACA N-(5-hydroxypentyl), AB-CHMINACA M2, AB-FUBINACA M3, UR-144 N-(5-hydroxypentyl) and PB-22 N-(5-hydroxypentyl) metabolites) in hair. These methods were applied to hair samples received for workplace and criminal justice testing.

Methods: An LC-MS/MS method was developed to detect 15 synthetic cannabinoids in hair. Sample preparation included hair washing followed by drug extraction from the hair with methanol in a 1-hour CEM MARS™ 6 microwave extraction at 95ºC. The extract was then dried down and reconstituted in methyl tert-butyl ether (MTBE) followed by a liquid-liquid extraction with deionized water. The MTBE was then evaporated to dryness and reconstituted in 250 µL of 90:10 methanol:deionized water. Instrumental analysis was performed on an AB-Sciex 5500 QTRAP® with a Phenomenex Kinetex® 5 µm Biphenyl 100 Å 100 x 3.0 mm column and gradient elution with 0.1% formic acid in deionized water, and 0.1% formic acid in acetonitrile. The screening cutoff was set to 10 pg/mg of hair. Samples identified for the parent drug were sent to confirmation for the metabolite.

An LC-MS/MS confirmation method was developed to detect 12 synthetic cannabinoid metabolites in hair. Sample preparation included hair washing followed by drug extraction from the hair with methanol in a 2-hour CEM MARS™ 6 microwave extraction at 95ºC. The extract was then dried down, reconstituted in MTBE followed by liquid-liquid extraction with 0.1 M ammonium formate buffer (pH 3.5). The MTBE was then evaporated to dryness and reconstituted in 100 µL of 50:50 methanol:deionized water. Instrumental analysis was performed on an AB-Sciex 6500+ QTRAP® with a Phenomenex Kinetex® 1.7 µm Biphenyl 100Å 150 x 2.1 mm column and gradient elution with 2 mM ammonium formate and 0.2% formic acid in deionized water, and 2 mM ammonium formate and 0.2% formic acid in acetonitrile.

Results: An LC-MS/MS screen was developed for the detection of JWH-018, JWH-073, AM2201, UR-144, XLR-11, AB-PINACA, 5-fluoro AB-PINACA, AB-FUBINACA, ADBICA, ADB-PINACA, AB-CHMINACA, APINACA, PB-22, 5-fluoro PB-22 and BB-22 in hair.

An LC-MS/MS confirmation method was developed for the detection of JWH-018 N-(5-hydroxypentyl), JWH-073 N-(4-hydroxybutyl), JWH-073 N-(3-hydroxybutyl), AM2201 N-(4-hydroxypentyl), AB-PINACA N-(5-hydroxypentyl), APINACA N-(5-hydroxypentyl), ADBICA N-(5-hydroxypentyl), ADB-PINACA N-(5-hydroxypentyl), AB-CHMINACA M2, AB-FUBINACA M3, UR-144 N-(5-hydroxypentyl) and PB-22 N-(5-hydroxypentyl) metabolites in hair. Chromatographic separation was achieved for analytes with multiple isobaric hydroxy metabolites. Cutoff concentrations were set at 0.5 pg/mg for JWH-073, JWH-018, AM2201, PB-22, and AB-PINACA metabolites; 1.0 pg/mg for UR-144, APINACA, ADB-PINACA, ADBICA, AB-CHMINACA metabolites; and 2.0 pg/mg for AB-FUBINACA metabolite.

Positive samples were identified through the screening of criminal justice samples. The ratios of metabolite concentration to parent concentration were investigated. Samples were identified containing AM-2201, UR-144, XLR-11, AB-FUBINACA, AB-CHMINACA, PB-22, 5F-PB-22, AB-PINACA, 5F-AB-PINACA, ADBICA.

Conclusion/Discussion: LC-MS/MS methods for the screening of synthetic cannabinoid parent drugs and the confirmation of synthetic cannabinoid metabolites were developed. The predominance of parent compound in the hair allows for development of methods for detection of emerging synthetic cannabinoids on the market prior to the characterization of their metabolites and the commercial availability of metabolite reference standards.

POSTER

P102: Development and Validation of a Novel rFab Highly Sensitive Homogeneous Immunoassay for the Detection of Amphetamine in Oral Fluid Collected by the Quantisal Family Oral Fluid Collection Device

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Immunalysis Corporation, Pomona, CA, U.S.A

Background/Introduction: Rising abuse of prescribed and illicit amphetamines constitutes a serious public health concern. Amphetamine was discovered over 100 years ago and is a synthetic derivative of the phenylethylamines group. Therapeutic applications are restricted to the treatment of attention-deficit/hyperactivity disorder (ADHD) and narcolepsy.   Due to its strong stimulating, appetite-suppressing and euphoric effect in high doses, it is widely abused with a serious risk of addiction. Oral fluid is a viable matrix option for testing drugs of abuse and offers many advantages over other matrices. The main benefits are the noninvasiveness and simplicity of collection, meaning it can be performed almost anywhere under direct supervision with minimum risk of alteration.

Synthetic Enzyme Fragment Immunoassay (SEFRIA) is a highly sensitive competitive homogeneous enzyme immunoassay with a much lower detection limit, therefore, ideal for oral fluid samples. This advantage over traditional G6PDH –based homogeneous enzyme assays stems from its unique methodology, which employs the reassociation of two inactive enzyme fragments to form an active enzyme. This results in a low assay background with little matrix interference attributed to its longer wavelength characteristics

An added challenge is to manufacture a specific amphetamine antibody due to the structural similarity with a variety of synthetic structures such as "bath salts" that can easily cause a high number of false positive samples. The combination of a well-designed immunogen and recombinant fragment binding (rFab) technology allow us to obtain an antibody with higher specificity than commercially existing ones.

Objectives: The objective of this project was to develop and validate a highly sensitive SEFRIA immunoassay based on a newly developed amphetamine rFab for the rapid detection of amphetamines in human oral fluid collected by the Quantisal Family Oral Fluid Collection Device.

Methods: The assay was designed to detect amphetamine in oral fluid collected by the Quantisal Family Oral Fluid Collection Device in qualitative and semi-quantitative analysis at a 50 ng/mL cutoff. The SEFRIA Amphetamine assay was analyzed on the Beckman Coulter AU480 system. Precision, linearity, and method comparison were among the characterization tests performed to validate the assay. The precision was tested at ±25% and ±50% of the cutoff. The linear range from 20 – 200 ng/mL was established by drug recovery. The method comparison oral fluid samples were collected in Quantisal Collection Device and the samples were analyzed by the SEFRIA Amphetamine Oral Fluid Assay and LC-MS/MS.

Results: Precision concentration levels at -50% and -25% of the cutoff were negative, and concentration levels at +50% and +25% of cutoff were positive for both semi-quantitative and qualitative analysis, total sample n = 80. The linear range of the assay was 20 to 200 ng/mL. A total of 80 oral fluid samples previously analyzed by LC-MS/MS were tested for method comparison. The sensitivity, specificity and accuracy of the assay were found to be 100%, 100% and 100%, respectively.

LC-MS/MS (50 ng/mL)			
		(+)	(-)
SEFRIA Amphetamine OF Test Device (50 ng/mL)	(+)	40	0
	(-)	0	40

Conclusion/Discussion: A highly sensitive SEFRIA immunoassay based on novel amphetamine rFab has been developed for the broad detection of amphetamine in human saliva. When applied to authentic specimens the assay correlated well with LC-MS/MS results.

P103: Development and Validation of a Novel rFab Highly Sensitive Homogeneous Immunoassay for the Detection of Cocaine and Benzoylecgonine in Oral Fluid Collected by the Quantisal Family Oral Fluid Collection Device

Steven Martinez, **Guohong Wang***, Kim Huynh, Michelle Nguyen, Jialin Liu, Joseph Buechler, Phillip Tam, and Michael Vincent

Immunalysis Corporation, Pomona, CA, U.S.A

Background/Introduction: Cocaine is a potent addictive stimulant that is found in the leaves of the coca plant. It is widely used in medicine as a local anesthetic, but also used as a stimulant by drug abusers. Cocaine and its metabolites, benzoylecgonine (BE) and ecgonine methyl ester appear rapidly in oral fluid following all routes of administration. This factor, along with the ease and non-invasive sample collection, makes oral fluid matrix an ideal alternative to urine for testing of cocaine and its metabolites for recent use or impairment. The relationship between cocaine and BE concentration allows for probable interpretation of results in response to time of use. Greater cocaine concentration indicates recent use while equal or considerably higher concentration of BE, or only BE, establishes cocaine usage occurring within days of drug intake. Therefore, an immunoassay that can detect both cocaine and BE in oral fluid matrix is ideal for a greater window of detection of cocaine use.

Objectives: The objective of this project was to develop a sensitive homogeneous enzyme immunoassay capable of detecting cocaine and benzoylecgonine at low drug concentration levels in human oral fluid collected using the Quantisal collection device. Synthetic enzyme fragment immunoassay (SEFRIA) is a highly sensitive immunoassay with greater sensitivity than traditional G6PDH-based homogeneous enzyme immunoassays. The increased sensitivity makes the SEFRIA platform ideal for high-through-put oral fluid drug testing with low cutoff requirements. BE has a longer detection time than cocaine and ecgonine methyl ester in saliva, making it the preferred target analyte for cocaine oral fluid SEFRIA.

Methods: The assay was developed to detect cocaine and BE in oral fluid collected by the Quantisal collection device for semi-quantitative and qualitative analysis at a cutoff of 15 ng/mL, with controls at ±25% of the cutoff value. Cross-reactivity, precision, linearity and method comparison studies were part of the characterization tests performed in the validation of the assay.

Results: Semi-quantitative and qualitative precision testing of the assay at -50% and -25% of the cutoff value produced negative results and testing at +25% and +50% of the cutoff produced positive results. The total number of samples used for each precision level was n=80. The linear range of the assay is 6 to 60 ng/mL. Cocaine has a 100% cross reactivity with the assay. The test was validated using a total of 96 oral fluid samples that were previously tested by LC-MS/MS for method comparison. Sensitivity, specificity and accuracy of the assay were found to all be 100%.

		LC-MS/MS (15 ng/mL)	
		(+)	(-)
Benzoylecgonine OF Test Device	(+)	60	0
(15 ng/mL)	(-)	0	36

Conclusion/Discussion: A highly sensitive immunoassay has been developed for the detection of cocaine and benzoylecgonine in saliva with a cutoff of 15ng/mL for neat oral fluid and 3.75ng/mL for diluted oral fluids. Comparison of LC-MS/MS results with authentic specimens shows a 100% correlation with this newly developed immunoassay.

P104: Development and Validation of a New and Highly Sensitive Homogeneous Immunoassay for the Detection of 6-Acetylmorphine in Oral Fluid

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Introduction: Heroin is a highly addictive semi-synthetic opioid and is an illicit drug in the United States. In recent years, the use has increased significantly. Heroin is most commonly administered by injection, nasal insufflation, or smoking. It has an extremely short half-life (minutes) and rapidly converts to 6-acetylmorphine (6-AM) and morphine; therefore, heroin itself is not an ideal target analyte for immunoassay. Its main metabolite, 6-AM is not present in pharmaceutical opioids and is selected over morphine as a unique biomarker for the detection of heroin illicit use.

6-AM appears in saliva in 2-5 minutes after heroin administration either by intravenous or smoking, versus 12 – 24 hours in the urine matrix. This advantage of early detection of 6-AM in oral fluid matrix can indicate recent exposure which may correlate with impairment.

The Synthetic Enzyme Fragment Immunoassay (SEFRIA) is an optimal homogeneous enzyme immunoassay platform for immunoassays that require a low detection limit; therefore, it is ideal for an oral fluid matrix that requires a lower cutoff than a urine matrix. The SEFRIA technology is based on two inactive enzyme fragments; Enzyme Acceptor (EA) and Enzyme Donor (ED) re-associates to form an active enzyme. As the 6-AM concentration increases in the sample, more active enzyme is formed that creates a dose response relationship between the 6-AM concentration and assay signal.

Objective: The objective is to develop and validate a highly sensitive SEFRIA immunoassay for the detection of 6-AM in human oral fluid. A new 6-AM recombinant fragment antibody (rFab)-based immunoassay has been developed with highly improved specificity when compared with conventional monoclonal antibodies-based immunoassays.

Method: 6-AM SEFRIA immunoassay is a semi-quantitative and qualitative assay with controls set at 25% of the 4 ng/mL cutoff. Immunoassay reagents were placed on a Beckman Coulter AU480 chemistry analyzer with validated parameters to establish a multi-point calibration curve for the semi-quantitative analysis to generate a concentration values in ng/mL. A two-point calibration for qualitative analysis to generate positive result if value were above cutoff and negative result if value were below cutoff. The precision, linearity, and method comparison studies were tested to determine the performance of the assay.

Results: The semi-quantitative and qualitative precision were determined by analyzing the assay performance in oral fluid for 5 days, 2 runs per day in replicates of eight (n=80). Precision concentration levels at -50% and -25% of the cutoff were negative, and concentration levels at +50% and +25% of cutoff were positive for both semi-quantitative and qualitative analysis. The linear range of the assay was from 2 to 20 ng/mL by testing recovery of the target analyte in the linear range. A total of 40 human oral fluid samples collected in Quantisal Family Oral Fluid Collection Device and previously analyzed by LC-MS/MS were evaluated and the sensitivity, specificity and accuracy of the assay were found to be 100%, 100%, and 100%, respectively.

		Confirmation (4ng/mL)	
		N	P
SEFRIA	N	20	0
(4 ng/mL)	P	0	20

Conclusion: It is the first time to report that a highly sensitive SEFRIA immunoassay has been developed for the detection of 6-AM in human oral fluid at 4ng/mL as cutoff concentration and +/-25% as controls.

POSTER

P105: Development and Validation of a Highly Sensitive Homogeneous Enzyme Immunoassay for the Detection of Cannabinoids in Oral Fluid Collected by the Quantisal Family Oral Fluid Collection Device

Steven Martinez, Guohong Wang, **Kim Huynh***, Jialin Liu, Feng Liu, Phillip Tam, Gabriel Huacuja, and Michael Vincent

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Background/Introduction: Rapid legalization of marijuana across the globe presents a myriad of opportunities for different applications of a drug that was once seen as a social taboo. To this day, cannabis remains one of the most produced, used, and trafficked drugs in the world. With its legalization prompting an increase in use, a need for rapid, precise and accurate testing is key for monitoring marijuana patients and surveilling drug use and impairment in workplace, criminal justice and driving under the influence of drugs. Oral fluid matrix has several advantages over matrices such as urine and blood. Sampling of oral fluid is non-invasive and easy to collect; therefore, it can be performed under direct supervision in virtually any location, minimizing the risk of alteration.

Cannabinoids oral fluid homogeneous enzyme immunoassay (HEIA) allows for the high throughput testing that accommodates the rapid increase of cannabis use with the rise in legalization of the drug. The selection of optimal antibody is essential for immunoassay development. There are over 100 naturally occurring cannabinoid structures found in cannabis; therefore, a highly specific cannabinoid antibody was selected to allow for a broad cross reactivity to many cannabinoids prevalent in modern marijuana production and consumption.

Objectives: Based on an in-house developed antibody and drug-G6PDH pairing, an extremely sensitive cannabinoids immunoassay has been developed at a 4 ng/mL cutoff for neat oral fluid or 1ng/mL for buffer diluted oral fluid collected using the Quantisal collection device.

Methods: The assay was developed to detect delta-9-THC in oral fluid collected by the Quantisal collection device for semi-quantitative and qualitative analysis at a cutoff of 4 ng/mL, with controls at ±25% of the cutoff value. Cross-reactivity, precision, method comparison and linearity were part of the characterization tests performed in the validation of the assay.

Results: The linear range of the assay is 2 to 20 ng/mL. Qualitative and semi-quantitative precision testing of the assay at -50% and -25% of the cutoff produced negative results and testing at +25% and +50% of the cutoff produced positive results, total samples n=80. The assay cross reacts with 11-nor-9-carboxy-delta9-THC, 11-hydroxy-delta 9-THC, cannabinol, and cannabidiol at 114%, 53%, 57% and 11%, respectively. 80 oral fluid samples that were previously tested by LC-MS/MS for method comparison were used to validate the immunoassay. The sensitivity, specificity and accuracy of the assay were found to all be 100%.

		LC-MS/MS (4 ng/mL)	
		(+)	(-)
HEIA THC OF Test Device	(+)	40	0
(4 ng/mL)	(-)	0	40

Conclusion/Discussion: A highly sensitive immunoassay was developed for the detection of cannabinoids in human oral fluid with cutoff at 4 ng/mL for neat oral fluid or 1 ng/mL for diluted oral fluids. Comparison of LC-MS/MS results with authentic specimens shows a 100% correlation with this newly developed immunoassay.

Jorge Daniel Sandoval*, Margaux Garnier, Cindy Coulter, Christine Moore

Immunalysis Corporation, Pomona, CA

Background: The Agilent Ultivo LC/TQ with jet stream technology was designed for saving laboratory space while still performing high sensitivity analysis. The opiates drug panel, that includes Oxymorphone (OXYM), Morphine (MOR), Hydromorphone (HYM), Oxycodone (OXYC), Codeine (COD), Hydrocodone (HYC), and 6-Acetylmorphine (6-AM), are common analytes that are tested for in today's toxicology laboratories. The confirmation cutoffs used, which were proposed by SAMHSA, were 2 ng/mL for 6AM and 15 ng/mL for all others. The method was validated according to standard protocols that include bias and precision, carryover, interference, ion suppression and enhancement, limit of detection, lower limit of detection, dilution integrity, and process sample stability. The developed method for the above opiates listed is described.

Objective: To develop a method for analysis of multiple opiate analytes listed above using Agilent Ultivo LC/TQ and apply the method to authentic donor samples obtained with the Quantisal™ II oral fluid collection device.

Methods: A fully validated and previously published solid-phase extraction method was used for sample preparation and analysis was carried out using the Ultivo LC/TQ. The extracts were run in positive ESI mode with a 5μL injection on an Agilent Eclipse Plus C-18 2.1x50mm × 1.8μm column. Mobile phase A (MPA) consisted of 5Mm ammonium formate (pH 6.4) with mobile phase B (MPB) as methanol. MPB at time 0 is 15%, by 3 minutes B was held at 25%, and at 7 minutes B was held at 90% with a stop time of 10 minutes. The flow rate was constant at 0.270 mL/min and all of the gas flows, voltages and temperatures were optimized for the highest level of sensitivity and reproducibility. Transitions, qualifying transitions and ratios are as follows:

Analyte	OXYM	MOR	HYM	OXYC	COD	HYC	6-AM
Transition Qualifier	302.1>**227.1**, 198.1	286.2>**165.1**, 153	286.2>**185.1**, 157	316.2>**298.1**, 241.1	300.2>**215.1**,165.1	300.2>**199.1**, 171.1	328.2> **211.1**,165.1
Ratio ±20%	75.0	89.0	71.0	26.6	92.5	40.5	69.9

Results: Below: Validation parameters

Analyte	OXYM	MOR	HYM	OXYC	COD	HYC	6AM
Calculated LOD (ng/mL)	1.67	0.41	0.99	1.39	1.49	1.38	0.12
Bias % (cutoff)	1.07	-1.18	-0.54	2.05	2.60	1.34	2.46
Precision % (cutoff)	10.54	3.20	3.80	3.46	3.86	3.38	2.06
Ion Suppression/Enhancement$_{(High)}$ %	8.42	42.40	48.18	7.41	31.40	38.90	37.33
Stability (hrs)	72	72	72	72	72	72	72
Interference	None	None	None	None	None	None	None
Carry Over	None	None	None	None	None	None	None

In addition, 60 oral fluid samples were collected with the Quantisal™ II device and evaluated for OXYC on the Ultivo LC/TQ. 1 mL ± 10% of neat oral fluid was collected per A and B split pads and placed in the transportation buffer. The A and B concentrations for OXYC ranged from 8-187 ng/mL. In all cases the quantitative difference between A and B splits had a variation less than 15%.

Conclusion: An analytical procedure for the simultaneous determination of OXYM, MOR, HYM, OXYM, COD, HYC, and 6-AM in oral fluid has been developed using Agilent Ultivo LC/TQ. The method validation was carried out according to standard protocols and the cutoffs used were from proposed SAMHSA guidelines. The Ultivo LC/TQ has a relatively small footprint and reduces laboratory workspace needed for the instrument; it is sensitive and performs well in the detection of opiates in human oral fluid collected with the Quantisal™ II device.

POSTER

P107: Quantitative Evaluation of delta 9-THC in Simultaneous and Sequential Oral Fluid Collection

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Immunalysis Corporation, Pomona, CA

Background/Introduction: Drug testing in oral fluid which requires a second, split sample be collected currently occurs by either simultaneous collection or sequential collection. Since a typical collection takes between 2 and 10 minutes to complete, the time delay between sequential collections could play a role in equivalence of drug concentration. The Quantisal™ II device was developed to avoid sequential collections thereby eliminating potential sources of error. Randomized simultaneous and sequential sampling of oral fluid from cannabis users using both the Quantisal™ and Quantisal ™ II oral fluid collection device is described, and quantitative values presented.

Objective: To evaluate the Quantisal™ II compared to the Quantisal™ collection device when both are used to collect from the same donor. Randomized order of sampling from 113 donors compares the concentration of delta 9-THC when collected sequentially or simultaneously. Sequential testing is defined as Quantisal™ then Quantisal™ II or Quantisal™ II then Quantisal™ collection.

Methods: The Quantisal™ and Quantisal™ II were used to collect 1mL neat oral fluid (+-10%) on each pad. Samples were collected until the volume adequacy indicator turned blue. The GC-MS confirmation values of THC in Quantisal™ collected samples were compared to the average of the THC concentration from samples collected on pads A and B from Quantisal™ II. In all cases the quantitative difference between A and B splits had a variation less than 15%. The average of all samples collected first was compared to the average of all samples collected second.

Results: Overall oral fluid samples collected first in the sequential testing scheme have an average concentration for THC of 177 ng/mL and collected second the average concentration is 114 ng/mL, a decrease of 64.4% between the two collections. Oral fluid samples collected simultaneously from the Quantisal™ II pads A and B have an average concentration of 143 and 144 ng/mL respectively. The difference in concentration is negligible. Out of the 113 collections 93 had a higher concentration on the first collection and 20 had a higher concentration on the second collection. Out of the higher second collections the difference was between 1 and 9 ng/mL. A significant difference of 44 ng/mL with was seen in one case. In one extreme case the first collection had a concentration of 41 ng/mL and the second 2 ng/mL. Concentration ranges, averages and medians are listed in the table below.

	Results (ng/mL)			
	1st collection	2nd collection	Pad A	Pad B
Average	177	114	143	144
Median	43	26	42	39
Conc range Low	3	2	2	2
Conc range High	2188	1604	1532	1677

Conclusion/Discussion: All 113 simultaneous or sequential collections were confirmed positive at or above a 2 ng/mL cut off using a previously published fully validated procedure. It is understood B sample could potentially be tested up to a year after collection based on Federal workplace urine drug testing guidelines for split collection according to the Federal Register Vol. 82, No. 13 Monday, January 23, 2017. Having starting concentrations similar for A and B splits would allow for better interpretation of the B split if it is analyzed after storage. Recommended storage for Quantisal™ collected samples is 2-10ºC. All samples tested in this study were analyzed at the same time to determine which mode of collection provided similar starting results guaranteeing a true split collection. It is thought that oral contamination from drug use or length of time between sequential collections could cause the first collection to be higher in concentration as seen in the example comparing 41 ng/mL to 2 ng/mL. In cases close to the cutoff this could be a potential issue as concentrations degrade over time. Use of sequential collections should be done with caution. Simultaneous collections are demonstratively preferred when samples could potentially challenge cutoffs.

P108: Drug Concentrations in Split Specimens of Oral Fluid Collected with Quantisal II after 1 Year of Storage

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Background/Introduction: Federal workplace urine drug testing guidelines for a split collection state the second sample (B) can be stored up to a year in the event of a drug result challenge. According to the Federal Register Vol. 82, No. 13 Monday, January 23, 2017, the testing of a split (B) specimen for a drug or metabolite is not subject to the testing cutoff concentrations established. The HHS-certified laboratory is only required to confirm the presence of the drug or metabolite that was reported positive in the primary (A) specimen. Applying this guidance to oral fluid analysis, a collection device which simultaneously collects two samples of oral fluid was developed. The Quantisal™ II simultaneously collects two oral fluid samples and allows for analysis of the A sample with discrete, separate storage for the B sample. Following the appropriate Federal urine guidance, the B split sample should be sent to an outside SAMHSA certified reference laboratory for analysis.

Objective: To evaluate the unopened Quantisal™ II B split after 1 year of refrigerated storage conditions by a SAMHSA certified reference laboratory.

Methods: Quantisal™ II oral fluid samples were collected from self-reported drug users. The A split was analyzed when received by Immunalysis™, and the B split was immediately stored in refrigerated conditions at 2-8ºC. The A split samples were analyzed for delta 9-THC, cocaine (COC), benzoylecgonine (BZE), PCP, methamphetamine (METH), amphetamine (AMP), and methadone (MTD). All analytical methods were fully validated, and all results were reported above the limit of quantitation. After one year of storage, the corresponding B splits were sent to a reference laboratory and the targeted confirmatory testing was performed.

Results: Concentration results are listed in the table below. In all cases, the same drug was found present in split sample B as was originally found in sample A. The concentrations of drug(s) found in split Sample B were at similar concentrations as was originally found in sample A. Concentration differences are noted for THC and COC/BZE in- particular. The results show that the B split samples were still positive with similar drug concentrations to the original results when tested after a year of storage.

Sample ID	Results (ng/mL)						
	THC	COC	BZE	PCP	METH	AMP	MTD
4232A			22		5152	396	201
4232B			17.5		5175	408.5	192.6
4233A		206	335		3963	596	1687
4233B		215.1	247		3395	485.3	1586.7
5311A	75			107			
5311B	53.3			117.1			
5313A	8			27			
5313B	4.3			27.1			
5316A	322	11	6	41			
5316B	496	4.1	8	47.3			
5319A	20			63			
5319B	8.2			74.4			
5321A				225			
5321B				270			
5322A	8			24			
5322B	5			24			

Conclusion/Discussion: In all 22 analyses performed, the B samples collected with a Quantisal™ II collection device and stored unopened up to a year at 2-8ºC were still positive. Some loss is to be expected with THC and COC in oral fluid samples. In all cases, the presence of the analyte was reported. The study shows the Quantisal™ II meets requirements for the B sample stability for Federal workplace drug testing.

POSTER

P109: Alternative Forensic Matrices: Evaluation of Simplified Workflow for Drugs of Abuse Extraction from Nail Samples Prior to LC-MS/MS Analysis

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Background/Introduction: The testing of alternative matrices in forensic and/or clinical toxicology is gaining popularity, partly due to less invasive means of collection. Blood and urine testing are still by far more prevalent. However, traditional testing in combination with other matrices such as hair or nail can provide a more rounded picture of abstinence or abuse and associated timeframes. Solid matrix analysis by Liquid Chromatography/Mass Spectrometry (LC/MS) or Gas Chromatography/Mass Spectrometry (GC/MS) is generally more involved due to the necessity of multiple manual labour steps to convert the sample into an extractable form.

Objectives: The aim is to demonstrate workflow advantages for fingernail analysis from multi-sample matrix homogenization, extraction and analysis for a range of drugs of abuse classes.

Methods: Nail samples (10 mg) were subjected to dry micro-pulverization using the Biotage® Lysera bead mill homogenizer. Samples were then soaked in 1 mL of methanol prior to clean-up using ISOLUTE® SLE+ 400 µL capacity supported liquid extraction columns. Manual positive processing was compared to the Biotage® Extrahera™ automated sample preparation platform. LC-MS/MS analysis was performed using a Shimadzu Nexera UHPLC system coupled to an 8060 triple quadrupole mass spectrometer. Multiple reaction monitoring (MRM) transitions were selected using the most intense precursor ions. LC mobile and stationary phases were selected based on analyte retention, resolution, symmetry and MS signal to noise. Final chromatography was performed using a Restek Raptor Biphenyl HPLC column with a combination of 2 mM ammonium formate and 0.1% formic acid as additives in both the aqueous and organic mobile phases.

Results: A typical panel of drugs of abuse including amphetamine and related analogues, ketamines, benzodiazepines, Z-drugs, cocaines, opiates, fentanyls and buprenorphines were investigated.

Initial nail extraction comparing dry and direct solvent-based homogenization was investigated. Due to the hard nature of the matrix better sample pulverization was achieved using dry samples. Nail extracts were then soaked in solvent and mixed further for effective drug transfer into solution. Methanolic solutions were selected for optimum solubility and drug transfer along with pH modification to aid subsequent extraction methodologies. Sample clean-up was performed using ISOLUTE SLE+ supported liquid extraction columns. Comparison of streamlined methodology loading the neat nail homogenate versus pre-concentration prior to loading was fully investigated. SLE optimization investigated loading pH and extraction solvent combinations from a recovery and suppression standpoint.

Screening results indicated that in order to get the best extraction of multi-class drugs of abuse ISOLUTE SLE+ extraction required an aliquot of 95/5 dichloromethane/isopropanol DCM/IPA (v/v) followed by a subsequent aliquot of methyl tert-butyl ether MTBE. The majority of analytes delivered extraction recoveries > 80% with all analytes demonstrating RSDs < 10%.

Calibration curves were constructed using fingernail samples spiked between 1-1000 pg/mg. Results demonstrated excellent linearity and coefficients of determination (r^2) greater than 0.99 for all analytes. LoQs were typically < 1 pg/mg for the majority of analytes in the panel. Sub 10 pg/mg levels were achieved for all other target analytes. Full comparison of the direct and pre-concentration extraction methodologies in terms of recoveries, RSDs, signal response, calibration curves and LOQs showed similar performance for most analytes. BZE recovery was substantially different between the methods. Extrahera processing allowed an automated extraction procedure directly comparable to offline positive pressure processing. However, in all cases the desired levels were met using both methodologies. Full details of the optimized workflow will be demonstrated.

Conclusion/Discussion: A simplified workflow for the extraction and analysis of multi-class drugs of abuse from fingernail samples has been demonstrated. This is beneficial as it reduces the number of steps necessary for traditional nail analysis. Fingernail analysis is a less invasive technique for anaylsing drug use than more conventional matrices.

POSTER

P110: Simplified Sample Preparation for Low Level Determination of Cannabis Use from Hair Samples Prior to LC-MS/MS Analysis

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Background/Introduction: Hair analysis is growing in popularity due to the non-invasive nature of the sample collection. Although not used routinely as per other matrices such as blood or urine, it does have advantages in that the matrix can indicate prolonged drug exposure. This can provide valuable information with respect to therapeutic drug regimens or in abused drug abstinence cases. Sample preparation for hair analysis is often lengthy involving multiple manual labour steps.

Objectives: The objectives of this research were to demonstrate workflow advantages for hair analysis; from matrix homogenization, extraction and analysis. Furthermore, the workflow will be demonstrated for application to the low levels required for cannabis testing from this matrix.

Methods: Hair samples (20 mg) were subjected to micro-pulverized extraction in methanol using the Biotage® Lysera bead mill homogenizer. Methanolic extracts were cleaned-up using supported liquid extraction: ISOLUTE® SLE+ in 400 μL capacity 96-well plates or equivalent columns. Liquid chromatography-mass spectrometric analysis was performed using a Shimadzu Nexera UHPLC system coupled to an 8060 triple quadrupole mass spectrometer. Multiple reaction monitoring (MRM) transitions were selected using the most intense precursor ions in positive or negative mode. LC mobile and stationary phases were selected based on analyte retention, resolution, symmetry and MS signal to noise. Final chromatography was performed using an ACE 2 C18 UHPLC column with a combination of 0.01% acetic acid as an additive in both the aqueous mobile phase and methanol as the organic eluent.

Results: A panel of cannabinoids and metabolites were investigated: cannabinol (CBN), cannabidiol (CBD), Tetrahydrocannabinol (THC), 11-Nor-9-carboxy-Δ^9-tetrahydrocannabinol (THC-COOH), 11-Hydroxy-Δ^9-tetrahydrocannabinol (THC-OH), $\Delta 9$-tetrahydrocannabinolic acid-A (THCAA).

Method development required investigation of LC mobile phases to achieve good chromatographic performance along with providing maximum positive and negative ion sensitivity. In this case a weakly acidic mobile phase utilizing 0.01% acetic acid in aq and organic solvent was selected. Previous work has identified the use of ammonium fluoride as an additive to maximize positive/negative ion sensitivity. However, in this case the acetic acid mobile phase yielded better performance.

Initial hair extraction was investigated to determine maximum matrix/solvent proportions. To achieve direct homogenization of the matrix in solution a 20 mg sample was selected with 1 mL of organic solvent. Methanol with or without pH modification was chosen for the extraction solvent, due to hair swelling ability allowing effective analyte release from the matrix.

Sample clean-up was performed using supported liquid extraction. A range of solvents were investigated from a recovery and suppression standpoint. Screening results indicated tertiarybutylmethylether (MTBE) to be the best performing solvent. In order to reach Society of Hair Testing LoQs we compared methanolic extract pre-concentration prior to extraction with direct extraction of the methanol hair extract. Typical recoveries were > 60% with corresponding RSDs < 10%. A range of extraction solvents were applicable depending on the exact panel required.

Calibration curves were constructed using hair spiked between 0.1-200 pg/mg. Results demonstrated good linearity and coefficients of determination (r^2) values greater than 0.99 for all analytes. Similar performance was achieved using SLE+ in the 400 μL capacity column as well as the high throughput 96-well plate formats. LoQs were below required SoHT guidelines for both screening and confirmation. THC-COOH achieved the 0.2 pg/mg levels set out.

Conclusion/Discussion: Hair matrix analysis is manually involved and complex to perform. Here we demonstrated simplified workflows for hair analysis utilizing simultaneous sample pulverization and subsequent extraction in column based format. Optimized cleanup allowed for the low-level detection of all cannabinoid metabolite tested achieving low or sub picogram levels as dictated by SoHT guidance.

P111: Tip-on-Tip Solid-Phase Extraction of Viscous Oral Fluid Samples

William E. Brewer* and Evan DiVirgilio

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Background/Introduction: Dispersive pipette extraction (DPX) tips have been commercialized and used for analyzing oral fluid for several years. Recently, we presented an improved product for comprehensive analysis of drugs in oral fluid that utilized mixed-mode strong cation exchange (SCX) and weak anion exchange (WAX) sorbent. In this study, we report a novel method for extracting drugs from viscous oral fluid samples. Pooled samples from a forensic/clinical laboratory that were unable to be processed because of clogging their existing sample preparation procedure were successfully analyzed using a new method referred to as tip-on-tip solid-phase extraction (SPE). The SCX sorbent is mixed loosely in the sample well, and the mixing is more reproducible because there is no clogging (if passing the sample through a porous frit of a SPE cartridge or DPX tip). We report recovery studies of spiked Oral-Eze buffer solutions, and report results of extractions utilizing this novel tip-on-tip SPE method.

Objectives: The objective of this study is to develop a rapid and automated method for analyzing comprehensive drugs (40 drugs including opiates, opioids, benzodiazepines, amphetamines, and antidepressants) in oral fluid using a novel tip-on-tip SPE method. In this study, difficult oral fluid samples that were considered too viscous to process with a conventional SPE product were used to verify the utility of this new method. In addition, recovery studies using various amounts of sorbent are shown.

Methods: The oral fluid samples (600 µL) were mixed loosely in a well plate on a shaker (or using wide bore tip mixing). In this manner, the sorbent could reproducibly mix with the viscous samples and allow for optimal binding. The solutions were mixed for 10 min on a Hamilton Nimbus96 liquid handler. After mixing, the solutions were transferred (by over-aspirating the solution with air to collect all of the solution) with the sorbent (by mixing) to 300 µL filtration tips on a vacuum block outfitted on the deck of the Nimbus96 system. After collecting the sorbent and drying in the filtration tips, the sorbent was washed with 300 µL of 10% methanol, then dried under vacuum. After drying for 5 min, 300 µL of 4% NH4OH in methanol were aspirated into 300 µL tips, inserted into the filtration tips on the vacuum block, and then the tips, tip-on-tip, were moved over the elution well plate, and the elution solvent was dispensed and pushed through the filtration tips and collected in the well plate. The solutions were solvent evaporated in reconstituted in 125 µL 10% methanol in water.

This method was used to process spiked Oral-Eze buffer solutions for recovery, linearity and LOD/LOQs. This method was also used for analysis of pooled samples from a forensic laboratory, with all samples failing analysis due to clogging issues. All analyses were performed using a SCIEX 6500+ triple quadrupole MS system coupled to an Agilent 1260 LC system equipped with an Agilent C18 column (Poroshell EC-C18 2.1 x 50 mm, 2.7 µm).

Results: Initial tests using the tip-on-tip extractions were performed with various amounts of sorbent (SCX and WAX). It was determined that 9 mg SCX sorbent provided recoveries greater than 80% recoveries for most of the drugs, providing linearities from as low as 0.0625 ng/mL to as high as 500 ng/mL, with linear regressions greater than 0.99 for all drugs. LODs ranged from about 0.05 ng/mL for fentanyl to 0.5 ng/mL for opiates, benzodiazepines and THC. Most of the compounds were extracted with less than 15% RSDs using this novel method.

Conclusion/Discussion: This study demonstrates a rapid, efficient and automated method for analyzing comprehensive drugs in oral fluid using a novel tip-on-tip SPE method. Difficult sample solutions that have failed analyses by a forensic/clinical laboratory were able to be readily processed.

POSTER

P112: Sensitive Analyses of Opiates and Opioids in Whole Blood Using New Tip-on-Tip Technology with LC/MS/MS

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Background/Introduction: LC/MS systems have made a significant impact on sample preparation requirements in forensic toxicology. In particular, highly sensitive LC/MS triple quadrupole instruments allow for low volumes of sample solutions, even when trying to achieve very low detection limits. In addition, the ability of HPLC separations minimizes the needs for rigorous extraction processes to purify samples for analysis. In this presentation, we demonstrate an automated protein precipitation method that provides rapid and sensitive analyses of opiates and opioids in whole blood. The automated procedure uses a novel tip-on-tip filtration method to readily process the samples.

Objectives: To develop a rapid and automated extraction method for opiates and opioids in whole blood.

Methods: Blank whole blood samples (from Utak) were spiked at various concentrations of opiates and opioids, including morphine, oxymorphone, hydromorphone, codeine, oxycodone, hydrocodone, 6-MAM, tramadol, meperidine, fentanyl, and methadone (spiked using a mix of single standards ordered from Cerilliant). Using just 0.2 mL of whole blood, the samples were added directly to well plates containing 100 mg of anhydrous $MgSO_4$ contained in 600 µL of acetonitrile. The samples were placed in a shaker and mixed for 5 min. After mixing, a wide bore tip aspirated 300 µL of the supernatant. Then the tip was moved and positioned into a 300 µL filtration tip, making an air tight seal, then the tip-on-tip device was placed over a well plate, and the solution was dispensed into the well plate. The wells were solvent evaporated using nitrogen and heat and reconstituted using 125 µL of 10% methanol.

All analyses were performed using a SCIEX 6500+ triple quadrupole MS system coupled to an Agilent 1260 LC system equipped with an Agilent C18 column (Poroshell EC-C18 2.1 x 50 mm, 2.7 µm). All extractions were performed using a Hamilton Nimbus96 liquid handler.

Results: The automated protein precipitation and tip-on-tip extractions were performed in less than 10 min, processing up to 96 samples simultaneously. Recoveries ranged from 76% (oxymorphone) to 125% (meperidine, which showed significant ion suppression). Samples were spiked at concentrations ranging from 0.1 ng/mL to 5.0 ng/mL. Limits of detection were for all compounds were found to be 0.1 ng/mL (and 0.05 ng/mL for fentanyl). LOQs were approximately 0.2 ng/mL for all of the drugs except fentanyl, which was 0.1 ng/mL. All linear regression values were greater than 0.99.

Matrix effects were also studied. With the exception of meperidine, all matrix effects were less than 20%. Additional studies are being performed to resolve the matrix effects for meperidine.

Conclusion/Discussion: This study demonstrates a rapid, efficient and automated method for analyzing opiates and opioids in whole blood. Further validation studies will be performed through collaboration with a forensic toxicology laboratory.

POSTER

P113: Withdrawn

P114: Development of a New Homogeneous Enzyme Immunoassay for the Detection of Opiates in Human Oral Fluid.

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Background/Introduction: With increasing popularity of urine adulterants and the issues associated with the collection and analysis of urine samples, oral fluid is gaining acceptance as the sample matrix for drug of abuse testing. SAMHSA 2015 proposed guidelines included additional opiates (codeine/morphine, oxycodone/oxymorphone, and hydrocodone/hydromorphone) to the SAMHSA panel for oral fluid testing. A separate assay for oxycodone and oxymorphone is currently available.

Objectives: The objective of the study was to develop a new homogeneous enzyme immunoassay for the qualitative determination of opiates (morphine, codeine, hydrocodone, and hydromorphone) in one assay in human oral fluid using the CEDIA™ technology.

Methods: CEDIA™ technology is based on the bacterial enzyme β-galactosidase which has been genetically engineered into two inactive fragments, Enzyme Acceptor (EA) and Enzyme Donor (ED). These fragments spontaneously re-associate to form an active enzyme. In the absence of analyte from the sample, the specific antibody binds to the ED-drug conjugate causing a decrease in enzyme activity. The free drug in the sample will compete for the limited number of antibody binding sites, making the ED-drug conjugate available for complementation to form an active enzyme. This phenomenon creates a direct relationship between the drug concentration in oral fluid and enzyme activity. The enzyme activity is then determined spectrophotometrically at 570 nm. The performance of the assay was evaluated on the Beckman Coulter™ AU680™ analyzer.

The CEDIA™ Opiates OFT assay uses a 30 ng/mL cutoff calibrator with controls at ± 25% of the cutoff. The assay consists of two lyophilized reagents and liquid ready-to-use calibrators and controls. Precision, cross-reactivity, interference, and method comparison studies were carried out to determine the overall performance of the assay. For the method comparison study, oral fluid samples were tested with the immunoassay and were confirmed by LC-MS/MS.

Results: Proof-of-concept studies demonstrated that the new CEDIA™ Opiates OFT assay detects all four opiates (morphine, codeine, hydrocodone, and hydromorphone) at 100% cross-reactivity. A precision study using the cutoff calibrator and ± 25% controls generated within-run and total-run precision (CV) ≤ 2.5%. The method comparison results demonstrated excellent agreement between the immunoassay and LC-MS/MS. No significant interference was observed, and the assay demonstrated minimal cross-reactivity to other concomitantly taken drugs.

Conclusion/Discussion: The preliminary data on the proof-of-concept CEDIA™ Opiates OFT assay demonstrates excellent precision, specificity, and sensitivity to all four opiate drugs (morphine, codeine, hydrocodone, and hydromorphone) without any significant cross-reactivity to other commonly abused drugs.

NOTE: The assay is currently in development and is not approved by FDA.

Keywords: CEDIA, OFT, morphine, codeine, hydrocodone, hydromorphone.

POSTER

P115: Stability of Mitragynine and 7-Hydroxymitragynine

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Background/Introduction: Mitragynine and 7-hydroxymitragynine are the psychoactive compounds present in kratom. Kratom has dose-dependent physiological and psychological effects with low doses producing stimulant effects and higher doses producing opiate-like effects. Due to kratom's legal status, ease of access, and growing popularity, the chemical properties of mitragynine and 7-hydroxymitragynine deserve further investigation. Drug stability can influence many factors including bioavailability, absorption, analytical, and pre-analytical factors, including storage and specimen integrity. Information regarding the stability of mitragynine and 7-hydroxymitragynine is relatively sparce. Mitragynine and 7-hydroxymitragynine were previously reported to be unstable in simulated gastric fluid (pH 1.2) but stable in simulated intestinal fluid (pH 6.8). Moreover, certified reference material producers recommend that 7-hydroxymitragynine is stored at -80°C in strongly alkaline media due to its unstable nature. To date, the pH and temperature-dependent variables that influence the stability of the *mitragyna* alkaloids have not been systematically evaluated.

Objectives: The goal of this study was to evaluate the pH and temperature-dependent stability of mitragynine and 7-hydroxymitragyine in aqueous media. In this short-term stability study, a wide range of pH (2–10) and temperature (4-80°C) were used, and degradation products were identified where possible.

Methods: Liquid chromatography-quadrupole/time of flight-mass spectrometry (LC-Q/TOF-MS) was used to evaluate the pH and temperature-dependent stability of mitragynine and 7-hydroxymitragyine. Dilute acid (pH 2) and aqueous buffers (pH 4, 6, 8, 10) were fortified with mitragynine or 7-hydroxymitragynine to achieve a final concentration of 2000 ng/mL. Solutions were maintained at 4°C, 20°C, 40°C, 60°C and 80°C and aliquots of solution were removed and analyzed over a period of eight hours. Immediately following fortification, aliquots (n=2) of each sample were removed and analyzed to establish T_0 (0% loss). Sampling intervals following T_0 were 0.5, 1, 2, 3, 4, 5, 6, 7 and 8 hours. Decreases in concentration were monitored using a previously validated LC-Q/TOF-MS method. Limits of detection for mitragynine and 7-hydroxymitragynine were 0.5 and 1 ng/mL, respectively. Half-lives for each species under various conditions of temperature and pH were generated from rate plots, and accurate mass and MS^2 spectra were used to identify degradation products where possible.

Results: Mitragynine and 7-hydroxymitragynine were unstable in extremely acidic (pH 2) and basic conditions (pH≥ 8), particularly at elevated temperatures (60°C and 80°C). Both compounds were found to be stable at more moderate conditions (~pH 6) and at refrigerated or frozen temperatures (≤4°C). Mitragynine and 7-hydroxymitragynine were stable at moderate temperatures (20°C and 40°C) for at least 8 hours. Half-lives were determined when a significant loss (>20%) was observed over consecutive measurements. In addition, two major degradation products of mitragynine under basic (*m/z* 385) and acidic (*m/z* 397) conditions were identified.

Conclusion/Discussion: The short-term stability of mitragynine and 7-hydroxymitragynine was pH and temperature-dependent. While both compounds were stable at moderate pH and low temperature, instability was observed at extreme pH. Significant acid-lability was observed, particularly at elevated temperature. These findings should be considered during mitragynine analysis, particularly during extraction and sample preparation steps. Additional studies to evaluate the long-term stability of *mitragyna* alkaloids is needed, in particular their stability in biological specimens.

POSTER

P116: Urinary pH: Effect on 7-Aminoclonazepam Stability and Immunoassay Screening

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Background/Introduction: Urine is a commonly collected matrix for workplace drug testing and for drug rehabilitation centers as it shows prior and chronic use of drugs. After collection, urine samples are sent to a clinical or forensic laboratory for analysis. The transportation process may take hours or days depending what laboratory is contracted to conduct the analysis and their distance from the collection site. In rare instances, such as samples collected abroad from military members, there can be upwards of a week before they arrive at the laboratory. During this time, samples often sit in uncontrolled temperature environments with elevated temperatures, thus increasing the pH of the specimen. Measuring pH is one of the adulteration checks set by Substance Abuse and Mental Health Services Administration (SAMHSA). Urinary pH averages in the slightly acidic range (pH 5 - 6), and exceeding a pH threshold of 9 is a trigger for an invalid sample.

In addition to concerns regarding sample pH, drug stability is a concern for laboratories performing analysis, especially during quantification of analytes. The analyte of interest to this study, 7-aminoclonazepam (7-AC) has been shown to be unstable in urine at a pH of 6 with sodium fluoride.

Objectives: The purpose of this study is to investigate if differing urinary pH affects the degradation of 7-AC in refrigerator and freezer conditions over a 30-day period. Additionally, this study is designed to determine if elevated pH levels affect the sensitivity of antibody screening techniques.

Methods: A pooled urine sample was split into three bottles and spiked with 7-AC (1000 ng/mL), 7-AC (1000 ng/mL) and diazepam (500 ng/mL), or no drug. The aliquots were stored at room temperature and allowed to increase in pH. Aliquots were withdrawn, on days 0, 1, 2, 3, 5, 7, 10, 14, 21 and 30 and their pH was measured. The samples were then stored at 4 °C, -20 °C or -80 °C pending analysis. After 30 days of storage, aliquots were screened on EMIT and ELISA, and quantified using hydrolysis and protein precipitation followed by LC/MS/MS. The aliquots that were stored at -80 °C from day 0 were used to determine an initial concentration for the 30-day study.

Results: The pH of the three bottles did not increase in the same manner over time despite having been taken from the same original source. This indicates variance due to the microenvironment of each bottle. The immunoassay results were negative for the bottle only spiked with 7-AC at all pHs and storage conditions. A majority of the aliquots for this bottle quantified above the manufacturer cutoffs. All aliquots screened positive for the bottle spiked with both 7-AC and diazepam. 7-AC had a concentration decrease greater than 20% at all pHs for the -20 °C storage. Under 4 °C storage, the 7-AC was inconsistent in percent degradation if spiked with diazepam or spiked alone. There was sudden degradation of 7-AC of almost 70% in a set of -20 °C aliquots, indicating another mechanism of degradation occurs in addition to pH. Diazepam was stable for both storage conditions under all pHs.

Conclusion/Discussion: A less considered stability factor is the pH of the matrix upon storage. While 7-AC is an unstable drug over 30 days regardless of storage conditions, it appears that freezer conditions had more degradation at lower pHs than fridge conditions. As pH increases, fridge and freezer conditions converge in their instability, showing pH has some degree of effect, however it does not fully explain the instability of 7-AC in urine.

Keywords: urine pH, stability, 7-aminoclonazepam

P117: A Dilute-and-Shoot LC-MS/MS Method for Antipsychotics in Urine

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Background/Introduction: Estimates of adherence to antipsychotics have been made from patient interviews, pill counting, and blood testing. A number of methods for the analysis of antipsychotics in blood have been reported for both therapeutic drug monitoring and postmortem testing for toxicity. This report details a dilute and shoot method for the analysis of 19 different antipsychotics and metabolites in human urine.   The method takes advantage of earlier reports demonstrating unique, prevalent urine metabolites for aripiprazole, brexpiprazole, haloperidol and lurasidone to enhance sensitivity for these drugs. With a fast analysis time (<5 minutes) and minimal sample preparation, this method has been used for quantitation of antipsychotics and relevant metabolites in urine. Finally, this method has been used to test samples for over a year with the results summarized in this report. While further improvements are certainly possible, this method is selective and sensitive for this group of important compounds.

Objectives: To develop and validate a novel, rapid dilute-and-shoot method for the analysis of antipsychotics and relevant metabolites in urine. Further, to illustrate the range of values that can be observed from a population of patients prescribed the drugs of interest to this report.

Methods: Samples were diluted 10X with a solution of internal standards dissolved in mobile phase A before they were analyzed by LC-MS/MS on a Agilent system comprised of a 1290 chromatography stack with a Kinetex Phenyl-Hexyl, 2.1 × 50mm, 1.7μm; (Phenomenex Part Number: 00B-4500-AN) and a 6490 MS/MS unit. A gradient of 5 mM ammonium formate with 0.1 % formic acid in 10% methanol/water (mobile phase A) and 5 mM ammonium formate with 0.1% formic acid in methanol (mobile phase B) was carried out as follows (time/%B): Initial/10%, 0.60/30%, 3.00/65%, 3.10/90%, 3.40/90%, 3.45/10%, 3.70/10%. The flow rate was 600 μL/min. The injection volume was 2 μL. The validation of this method followed College of American Pathologist (CAP) and Clinical Laboratory Improvement Amendment (CLIA) guidelines.

Results: The calibration curves for all analytes were quadratic with either 1/X or $1/X^2$ weighting. The Limit of Quantitation (LOQ) was generally 5 ng/mL for parent drugs and/or low prevalence metabolites (e.g., aripiprazole or dehydroaripiprazole) or 25 ng/mL for drugs and metabolites that are more prevalent in urine. Matrix effects ranged from -75.10% for N-desmethylolanzapine to +86.90% for OPC 3952, a primary metabolite of brexpiprazole. Retention times under the conditions used herein ranged from 0.38 min for N-desmethylolanzapine to 2.43 min for lurasidone.

Normal ranges over a year do not appear to correlate with daily dose levels. For example, the median concentration of haloperidol was 514 ng/mL with daily dosing of 15 mg while the median for quetiapine was 87 ng/mL with daily dosing of 250-550 mg. The median for clozapine was the highest of all the drugs studied in this work at 1226 ng/mL while aripiprazole was the lowest at 17 ng/mL.

Conclusion/Discussion: The method reported here is a dilute-and-shoot method that only requires dilution with the selected internal standards solution and centrifugation before analysis. It is not dependent upon sample "cleanup" and is thus much faster than traditional blood methods in overall test time. An additional advantage of this method is the use of recently reported urine metabolites for several of these drugs. The use of these relatively prevalent metabolites enhances the sensitivity of this method for determining adherence to prescribed medications.

POSTER

P118: Unrolling the Challenges of CBD in the Drug Testing Laboratory

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Background/Introduction: Detecting the presence of 11-Nor-9-carboxy-Δ^9-tetrahydrocannbinol (carboxy-THC) is the targeted component of urine drug testing confirming marijuana use for a variety of legal and medical reasons. THC, the main psychoactive component, is just one of many cannabinoids found in marijuana. The non-psychoactive cannabinoid, cannabidiol (CBD), has gained a reputation as a natural remedy for a variety of medical conditions. This sudden rise in CBD use stresses the importance to definitively and selectively detect the use of marijuana. The impact of CBD use must be understood to mitigate the risk that CBD use will produce inadvertent-positive results for marijuana use. This is a complex, multifaceted concern. Even when using sound analytical techniques, discriminating CBD use from marijuana use remains challenging as unregulated CBD products are frequently contaminated with varying amounts of THC, exposing CBD users to coincidental THC. While the exposure to THC as a contaminant in CBD products is generally small, the exposure can be significant enough to result in a positive test for carboxy-THC. Inadvertent THC exposure from CBD products, like passive marijuana exposure, falls outside the intended goal of drug testing.

Additionally, savvy marijuana users are aware of the laboratory difficulties differentiating CBD use from marijuana use, and some are known to claim CBD use as a method to obfuscate positive test results.

Objectives: The laboratory set to develop a procedure to identify positive urine THC tests which result from CBD users inadvertently exposed to THC from impure CBD products.

Methods: A selection of CBD metabolites was synthesized in-house for use as analytical standards. Urine samples from over 200 donors claiming CBD use and samples from donors personally known to exclusively use CBD were analyzed to quantify CBD, 7-carboxy-CBD, THC, carboxy-THC (all released from phase II conjugates). Samples were initially processed using a two-step enzymatic and chemical hydrolysis. Analytes were partitioned into acetonitrile and the extracts were analyzed by UPLC-MS/MS positive electrospray ionization monitoring 3 transitions per analyte on a Sciex 6500+ QTRAP®. Chromatographic separation of the analytes was achieved using a 10 mM ammonium acetate, 0.1% formic acid / acetonitrile gradient through a Waters Acquity UPLC® HSS T3 column. The gradient started at a composition of 45% acetonitrile and stepwise ramped up to 95% over 3 minutes. The analytical methodology was fully validated. Lower quantitation limits for phase I metabolites were 5 ng/mL; LLOQs for parent drugs released from phase II conjugates were 0.6 ng/mL.

Results: Both CBD and 7-carboxy CBD were found at significant concentration in urine samples from users known and believed to be CBD users; 7-hydroxy CBD was also found in these samples but was not measured. Relative concentrations of the CBD metabolites varied significantly between donors. Samples from users known and believed to be exclusive CBD users possessed CBD metabolites at concentrations at least ten-fold higher in concentration than excreted THC metabolites. In these samples CBD levels ranged from 0.6 to 1700 ng/mL and 7-carboxy CBD levels ranged from 5 to 2900 ng/mL while carboxy-THC levels in these same samples ranged from none-detected to 110 ng/mL. Samples with near equivalent levels of CBD metabolites to THC metabolites or THC metabolites exceeding CBD metabolites were found to be not consistent with exclusive CBD use.

Conclusion/Discussion: Through the close quantitative evaluation of CBD metabolites relative to THC metabolites in a urine sample, the risk for false positive carboxy-THC determinations as a result of THC contaminated CBD products can be successfully mitigated in most cases. Inversely, donor claims of positive carboxy-THC test results stemming from CBD use can be disproven using the same analysis.

POSTER

P119: Serum Plasma Fentanyl Concentrations Increased in Rhesus Monkeys after Fentanyl-TT Conjugate Vaccine Administration

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Background/Introduction: Since 2006, the number of fatal and nonfatal opioid related overdoses has increased. Fentanyl, a synthetic opioid, has significantly contributed to this public health crisis. Currently, naloxone is the only Food and Drug Administration approved treatment for an opioid overdose. The National Institutes of Health has outlined several treatment strategies to address this epidemic, including the development of opioid-targeted conjugate vaccines or immunopharmacotherapies. These vaccines stimulate the immune system to produce high-affinity antibodies against the targeted opioid. Upon exposure, antibodies bind to the targeted opioid preventing it from crossing the blood brain barrier, blocking centrally mediated effects. Potential advantages of immunopharmacotherapies include high selectivity towards targeted opioids and a longer duration of action, thereby mitigating the need for repeated dosing. It has been previously demonstrated that a fentanyl vaccine can reduce fentanyl's behavioral effects. The study suggested that this was caused by the antibody sequestered fentanyl remaining in the blood thus increasing plasma fentanyl concentrations

As part of ongoing studies using the Scripps Research Institute fentanyl-tetanus toxoid (TT) conjugate vaccine conducted at Virginia Commonwealth University (VCU), plasma samples were collected from four adult male rhesus monkeys (*Macaca mulatta*) at 3, 10, 30, 100, 300 min, and 24 h after 0.018 mg/kg, intramuscular fentanyl administration before fentanyl-TT conjugate vaccination and then 5 weeks post-vaccination.

The animal maintenance and research were conducted in accordance with 2011 guidelines promulgated by the NIH Committee on Laboratory Animal Resources. The facility was licensed by the United States Department of Agriculture and accredited by AAALAC International. Both research and enrichment protocols were approved by the VCU Animal Care and Use Committee.

Objectives: To determine if fentanyl-TT conjugate vaccine significantly changes plasma fentanyl concentrations

Methods: Fentanyl was extracted from plasma using a solid phase extraction (SPE) technique. Briefly, 5ng fentanyl-d$_5$ was added to calibrators, quality controls and samples followed by phosphate buffer (pH 6.0). Samples were mixed, centrifuged and then extracted using SPEC MP3 SPE columns. Columns were conditioned with methanol and phosphate buffer (pH 6) followed by the samples. The columns were then washed with water and acetic acid and eluted with 78:20:2 dichloromethane/isopropanol/ammonia (v:v:v). Samples were evaporated under nitrogen and reconstituted with mobile phase for analysis.

The analysis was performed on a Waters Xevo TQD UPLC-MS/MS system controlled by MassLynx software (Milford, Massachusetts) with an Ultra Biphenyl 3 μm 50 x 2.1 mm column (Restek, Bellefonte, Pennsylvania). The mobile phase consisted of A: 20 mM ammonium formate in water and B: 20 mM ammonium formate in methanol. The gradient used at a flow rate of 0.6 mL/min was 0.00 to 1.5 minutes at 5% B, 1.5 to 3 minutes at 40% B, 3 to 3.5 minutes at 100% B, returning to 5% B at 3.6 minutes. The MRM transition ions (m/z) and corresponding collection energies (eV) in parentheses were fentanyl: 337>105 (36) & 337>188 (24) and fentanyl-d$_5$: 342>105 (36) & 342>188 (24). Total run time was 4.0 minutes.

Results: Peak plasma fentanyl concentrations following 0.018 mg/kg fentanyl (IM) administration occurred at 10 min (41 ng/mL) and was 136 ng/mL 5 weeks following the vaccine. Plasma fentanyl concentrations were significantly higher following vaccine administration at all time points. The fentanyl vaccine reduced central mediated effects.

Conclusion/Discussion: This study demonstrated plasma fentanyl concentrations were significantly higher in vaccine treated monkeys up to 5 weeks after a single vaccination. Peak 5-week post-vaccination concentrations were approximately 3 times higher than the peak pre-vaccination concentrations. Indicating that the administered fentanyl is being sequestered in the blood and is suggestive of reduced brain fentanyl concentrations.

POSTER

P120: Acetone Interference with Breath Alcohol Analysis

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Introduction: When quantifying ethanol in human breath, the presence of interfering volatile organic compounds (VOCs) has been a concern. Allegations of VOC interference resulting in elevated breath alcohol concentration is a well-known defense argument in trials concerning alcohol impairment.

Objectives: Not much information exists to substantiate this claim, especially when modern analytical techniques such as coupled infrared and electrochemical sensors are used for forensic measurement. Testing of human breath VOCs at reasonable concentrations was carried out on an Intox EC/IR II desktop evidential breath alcohol testing instrument.

Methods: Solutions of endogenously produced VOCs including methanol, isopropanol, acetone, and acetaldehyde with and without ethanol were prepared at forensically relevant concentrations spanning 0.010 to 0.100 g/100mL and tested. Fuel cell and infrared spectroscopy response curves were collected and compared against ethanol laden samples.

Results: The Intox EC/IR II breath alcohol testing instrument is designed to quantify ethanol-laden human breath. VOCs with molecular structures similar to ethanol such as methanol, isopropyl alcohol, and acetaldehyde elicit a diminished electrochemical response. The measured concentration of these VOCs is between 30% to 50% of the assayed solution value with and without ethanol. Acetone was not electrochemically oxidized to produce a response at any concentration.

Conclusions: Test conditions and instrument responses to various concentrations of VOCs with and without ethanol were compared. Instrument design features and testing protocols aim to prevent quantification of interfering VOCs, resulting in a device that is selective for alcohols. It should be noted that the presence of these VOCs on the breath in high enough concentrations to warrant a prohibitive alcohol concentration citation, would normally be associated with impairment.

POSTER

P121: Simple, Rapid, and Accessible Detection of Ethanol in E-Liquids by Fuel Cell BrAC Test

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Background/Introduction: Ethanol has been demonstrated to be a significant ingredient in electronic cigarette formulations (e-liquids). It is used as a thinning agent, as a solvent for flavoring chemicals and drugs, and as an intoxicant. In 2016, the Food and Drug Administration promulgated regulations requiring ingredient labeling for tobacco products extending to e-liquids. However, this has not diminished the rampant production and sale of e-liquids containing unlabeled ingredients. Humectants in e-liquids, e.g. propylene glycol (PG) and vegetable glycerin (VG) form small particles when aerosolized capable of transporting intoxicants deep into the lung. It has been suggested that vaping ethanol can interfere with roadside preliminary breath tests (PBTs). Law enforcement officers are equipped with fuel cell breathalyzers used for PBTs. Fuel cell detectors are electrochemical cells that oxidize ethanol to generate an electrical current measured to produce a breath-alcohol concentration (BrAC). The Intoxilyzer 800® (CMI Inc., Owensboro, KY) is equipped with a sampling cup that can be used to detect ethanol within a liquid.

Objectives: The objective was to evaluate the ability of the Intoxilyzer 800® to screen for ethanol in e-liquid formulations.

Methods: E-liquids were prepared using PG, VG, and 50:50 PG:VG with ethanol concentrations of 0, 0.25, 0.5, 1, 2, 5, and 10% *v/v*. Ten milliliter samples (n = 5) were measured using a fuel cell breathalyzer (CMI, Inc. Intoxilyzer 800®) BrAC test with sampling cup placed directly over the opening of a 20 mL scintillation vial for 2, 5, and 10 seconds to evaluate volatile acquisition time. The range of detection for the Intoxilyzer 800® is 0.000 g/210 L to 0.440 g/210 L. Neat methanol, isopropanol, acetone, mouthwash, and alcohol-free mouthwash were evaluated as potential interferences. The Intoxilyzer 800® was calibrated using CMI Ethanol Gas Standard 0.040 BAC.

Seventeen purchased e-liquids with concentrations of ethanol previously measured by headspace gas chromatography flame ionization detector (HS-GC-FID) were evaluated by the BrAC test described above. Using known ethanol concentrations in e-liquids a linear regression was calculated and compared with the HS-GC-FID results.

Results: Concentrations of 5% ethanol and higher resulted in the maximum BrAC reading >0.440 g/210 L for all acquisition times and humectant ratios evaluated. For known e-liquids below 5% ethanol, BrAC readings were slightly lower with e-liquids prepared in PG and highest with VG. As sample acquisition time increased, the BrAC measurement increased. Products evaluated with the Intoxilyzer 800® were determined to have twice the ethanol concentrations from HS-GC-FID. One false negative and three false positives were determined in the purchased e-liquids when they were transferred to a standard sampling container. Sample volume of the purchased e-liquids created significantly different BrAC readings and generated three false negatives and one false positive while in the original sample bottle. Of the possible interferences, neat methanol (0.275 ± 0.04 g/210 L), isopropanol (>0.440 g/210 L), and mouthwash (>0.440 g/210 L) all gave positive results in the Intoxilyzer 800®. Neat alcohol-free mouthwash (0.000 g/210 L) and acetone (0.000 g/210 L) both gave negative results in the Intoxilyzer 800®.

Conclusion/Discussion: In the field, a two second measurement with a fuel cell breathalyzer can screen for the presumptive presence of ethanol in e-liquid samples. False positives, false negatives, humectant ratios, sample volume, and acquisition time affects the reliability of this method. However, this simple procedure is a quick presumptive field screen for ethanol in advance of laboratory confirmation.

POSTER

P122: The Development of Extraction Methods to Evaluate Pharmacologically Active Compounds in Anticonvulsive Chinese Herbal Medicines by AccuTOF DART-MS

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Background/Introduction: Chinese herbal medicines (CHM) have been used in China for thousands of years, but the popularity of CHM has increased worldwide due to the relatively low cost, accessibility, and the perception that herbal or natural products have fewer adverse effects. In 2017, over 8 billion dollars was spent on herbal dietary supplements in the United States. Under an amendment to the Federal Food, Drug, and Cosmetic Act, herbal products fall legally under dietary supplements, which have lower quality standards than pharmaceuticals, potentially leading to adverse interactions. Synergistic and antagonistic interactions with western medications may also lead to toxicological emergencies that can be undetected in death investigations. Therefore, laboratories must be able to efficiently analyze cases in which CHMs are implicated.

Objectives: The aim of the study is to evaluate the differences between herbal remedies sold as anticonvulsive medicines, Gou Teng (plant), Tian Ma (plant), and Jiang Can (silkworm & fungus), purchased from shops in Beijing, China and Chinatown, New York. In order to achieve this, five extraction methods for anticonvulsants and other pharmacologically active compounds were compared: water, ethanol, microwave-assisted, ethanol:chloroform, and acid wash.

Methods: Herbal products were macerated, and 250 mg of product were used per extraction. For the water extraction, the herbal powder was soaked in 1.5 mL of hot water for 20 minutes, vortexed, centrifuged and supernatant was removed. The ethanol extract involved soaking the herbal powder in 1 mL of ethanol. For the microwave-assisted extraction, herbal powder was soaked in 65% ethanol, microwaved, and concentrated to 1 mL. The ethanol:chloroform extraction involved soaking the herbal powder in 1:1 ethanol:chloroform (v/v) followed by sonication and concentration to 1 mL. In the acid wash, herbal powder was soaked in acetic acid, followed by the addition of sodium hydroxide, hexane extraction, and reconstitution in 1 mL of ethyl acetate. The powdered herb and extracts were analyzed using a Jeol JMS T100LC AccuTOF Direct Analysis in Real Time-Mass Spectrometer (DART-MS) in positive and negative mode (n=5). Polyethylene glycol 600 in methanol and 1:1 methanol:dichloromethane was used for calibration in positive and negative mode respectively. Standard solutions of methamphetamine, cocaine, and nefazodone for positive and aspirin and furosemide for negative were used to check mass accuracy. Data analysis was performed using T.S.S Pro version 3.0 and Mass Mountaineer. Pharmacologically active compounds were identified based on exact mass within a 5 mmu range.

Results: The pharmacologically active compounds were consistent between sources for Tian Ma and Jiang Can. In the Gou Teng products, different pharmacologically active compounds were present between sources. Of the five extraction methods, no single extraction worked for all pharmacologically active compounds. The ethanol extraction was the most effective extraction for Gou Teng, while for Tian Ma, the water and microwave assisted extractions were most effective. For Jiang Can, the ethanol:chloroform was the most effective extraction.

Conclusion/Discussion: Differences in pharmacologically active compounds in herbal preparations, sold under the same name, could result from the use of different plant species. All methods evaluated were simple extractions using common solvents. The microwave-assisted extraction was the most effective method. These methods can be easily adopted for use with other popular natural products, such as Kratom and hemp.

POSTER

P123: Comparison of Various Sample Preparation Techniques for the Analysis of THC, Synthetic Cannabinoids, and Metabolites for LC-MS/MS Detection in Human Urine

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Background/Introduction: Tetrahydrocannabinol (THC) and synthetic cannabinoids are commonly encountered drugs in forensic toxicology, therefore, being able to extract these compounds and their metabolites is important. A variety of sample preparation techniques are available for the analysis of these compounds.

Objectives: The purpose of this study was to develop and compare the use of liquid-liquid extraction (LLE), solid phase extraction (SPE), and supported liquid extraction (SLE) for analysis using LC-MS/MS to identify and quantify THC, select synthetic cannabinoids, and their metabolites in human urine. Samples were analyzed using a Shimadzu Prominence Ultra-Fast Liquid Chromatograph (Shimadzu, Kyoto, Japan) with a 4000 Q-Trap Electrospray Ionization Tandem Mass Spectrometer (LC-MS/MS, SCIEX, Framingham, MA, USA).

Methods: All standards were prepared by spiking certified reference material (Cayman Chemical, Ann Arbor, MI, USA) into human urine which was donated following Institutional Review Board approved guidelines (Boston University School of Medicine, Boston, MA, USA). The LC-MS/MS was equipped with an XBridge® C18 3.5μm, 2.1 x 50 mm column (Waters Corporation, Milford, MA, USA) or a Kinetex® 2.6 μm C18 100 Å, 50 x 2.1 mm column (Phenomenex, Torrance, CA, USA). The LLE was carried out in 7-mL clear glass vials with PTFE-lined screw tops (Supelco, Bellefonte, PA, USA) using DI water (Millipore Milli-Q Ultrapure Type 1 Water System, Millipore Sigma, Burlington, MA, USA), glacial acetic acid (Acros, New Jersey, USA), methyl-tert-butyl ether (MTBE, Alfa Aesar, Ward Hill, MA, USA), hexane, and ethyl acetate (Fisher Chemical, Fair Lawn, NJ, USA) as solvents. The SPE was performed using a UCT Styre Screen® THC column (United Chemical, Bristol, PA, USA), acetonitrile (ACN), ammonium hydroxide (Fisher Chemical, Fair Lawn, NJ, USA), DI water, hexane, ethyl acetate, and glacial acetic acid. The SLE utilized Isolute® SLE+ columns (Biotage, Charlotte, NC, USA) with glacial acetic acid, hexane, ethyl acetate, and MTBE. All samples were reconstituted in starting mobile phase conditions consisting of 0.1% formic acid (Sigma-Aldrich, St. Louis, MO, USA) in DI water: ACN (70:30). Parameters assessed followed ASB Standard 036, Standard Practices for Method Validation in Forensic Toxicology, including recovery, limit of detection (LOD), limit of quantitation (LOQ), and calibration model.

Results: For all three techniques the linear dynamic range was determined to be from 1 ng/mL to 50 ng/mL. Recovery was highest for the LLE method, with all compounds having a recovery greater than 50%. The SPE method had the lowest recovery for all analytes. The LOQ for the SLE method was 0.5 ng/mL for 11-hydroxy-THC and 11-nor-9-carboxy-THC, 0.75 ng/mL for THC, AB-FUBINCA and AB-FUBINACA metabolite 3, and 1 ng/mL for AB-PINACA. LOQ for analytes extracted by LLE and SPE methods was 1 ng/mL for all compounds. THC and AB-PINACA had the lowest LOD, 0.25 ng/mL and 0.01 ng/mL, respectively, for the LLE method. 11-hydroxy-THC, 11-nor-9-carboxy-THC, and AB-FUBINACA metabolite 3 had the lowest LOD, 0.25 ng/mL, 0.1 ng/mL, and 0.01 ng/mL, respectively, for the SLE method. AB-FUBINACA had a LOD of 0.01 ng/mL for all three methods. Sample preparation required 1 hr for the SLE method while it took 2 hrs for the SPE method. The LLE method took 4 hrs to complete, consisting of a 2-hr freezing step in the middle of 2 hrs,

Conclusion/Discussion: In conclusion, three various sample preparation techniques were developed and validated using the ASB guidelines for method validation for the analysis of THC, synthetic cannabinoids, and metabolites. Although the recovery varied between all three methods, analysis can be completed for these compounds using any sample preparation technique. Looking at the time each method took, the amount of resources used, the cost, recovery, LOD and LOQ, the best sample preparation technique is the SLE method.

POSTER

P124: In Vitro Inhibition of Opioid Metabolism by Benzodiazepines

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Background/Introduction: Benzodiazepine drugs are frequently prescribed for the treatment of anxiety, insomnia, and alcohol dependence. In addition to their legitimate use they have become popular drugs of abuse, often in combination with opioids. Opiates pose a significant health risk alone but when taken in combination with other drugs there is the added risk of drug-drug interactions. The purpose of this study is to determine what benzodiazepines show potential for drug-drug interactions with the opioids buprenorphine, methadone and oxycodone. Inhibition of opioid metabolism could raise their concentrations to toxic levels or decrease the therapeutic effect of oxycodone by preventing metabolism to oxymorphone.

Objectives: The goal of this work was to establish the concentrations of benzodiazepine class drugs required to produce 50% inhibition (IC50 values) of *in vitro* CYPs or estimates from extrapolations when solubility was limiting.

Methods: Initially screens were conducted with human liver microsomes (HLM) to identify potential drug-drug interactions and estimate the concentrations of the drug needed for IC50 determination. Potential inhibitors at 3 different concentrations were incubated with HLM. The opioids were added along with or 15 minutes after the inhibitor and a source of NADPH (- and + pre-incubation, respectively) to test for time-dependent inhibition (TDI). The results of the screens were then used to identify which opioid/benzodiazepine combinations would be incubated with human CYPs to determine IC50 values. For presumptive IC50 concentrations below the solubility limit, a 10-concentration inhibition assay was performed with the respective CYP and opioid. If the presumptive IC50 was close to the solubility limit, a three-concentration assay was designed to permit potential extrapolation of an IC50. For several pairings, an IC50 > solubility limit was set.

Results: IC50 values or IC50 > solubility limits.

Conclusion/Discussion: IC50 results indicate that certain benzodiazepines are capable of significant inhibition of some CYP-mediated pathways. Extrapolating the *in vitro* values to *in vivo* values using the Cheng-Prusoff equation, $K_i = IC_{50} / (1+S/K_m)$, and an *in vivo* extrapolation equation, $AUC_i / AUC_n = 1 + [I]_{pl}/K_i$, demonstrates that several incubation combinations led to drug-drug interactions of potential clinical interest. Ratios of $AUC_i / AUC_n \geq 2$ indicate potentially significant inhibitions. The inhibition of CYP3A4/oxycodone metabolism by flurazepam exceeded this guideline. The inhibition of CYP3A4/buprenorphine metabolism by midazolam was below the guideline, but with pre-incubation the AUC_i / AUC_n ratio was high enough to warrant concern. The significant lowering of the IC_{50} with pre-incubation indicates that a metabolite of midazolam was responsible for the inhibition, at least in part.

P125: 6-Monoacetylmorphine, Under-Recovery and the Cold Truth of Pre-Analytical Variables

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Background/Introduction: Analysis of 6-monoacetyl-morphine (6-MAM) has been established as a marker for heroin use in biological matrices. The molecule is considered to be unstable under a variety of conditions (Zaitsu K., Miki A., Katagi M., et al. *Forensic Sci. Int.* 174. 2-3 Jan 30 (2008): 189-96) and losses after storage are generally considered to be related to degradation of the molecule. Under certain common laboratory conditions, it was found that 6-MAM could demonstrate under-recovery in human urine unrelated to stability. After allowing specimens to sit undisturbed at frozen or chilled conditions, specimens indicated up to 80% negative bias in analysis; recovery following appropriate mixing corrected for the bias.

Objectives: Evaluate the temperature and agitation-dependency on the recovery of 6-MAM in human urine specimens.

Methods: Specimens positive for 6-MAM were collected during the course of normal analysis. Human donor negative urine, synthetic urine (UTAK), 10% ACN in water, and DI water were fortified with 6-MAM to approximately 100 ng/mL. These samples were stored frozen prior to analysis. After being exposed to frozen conditions (<-10°C) for approximately 8 hours, the samples were removed and immediately placed on either a Hamilton Star deck for automated analysis or the bench for manual extraction. Specimens were aliquoted for testing after coming to room temperature; the samples were then mixed by inversion (1, 2 and 3 inversions) and re-aliquoted for extraction. The mixed samples were then left on the bench top for 3 days prior to re-testing.

 In a separate test, the Hamilton pipetting device was programmed to aspirate the sample from different depths of 2 mm and 7 mm from the liquid top as well as from the bottom of the tube.

Testing was performed by dilution of the samples with a 9:1 H2O:ACN mixture containing internal standards. Calibrators were prepared in synthetic urine and co-extracted with the samples. Diluted samples were injected onto a Waters Acquity system coupled to an API 5500 triple quadrupole mass spectrometer operating in positive electrospray mode. Three transitions were monitored for the analyte and 2 for the isotopically labeled internal standard.

Data was reduced in Analyst. All samples were measured against the calibration curve and results of the unmixed specimens were compared to the mixed specimens.

Results: A significant difference in the recovery of 6-MAM was shown between the unmixed and mixed specimens in almost all matrices. Of the fortified matrices, 10% ACN in water and DI water exhibited appropriate recovery of 6-MAM. Recently frozen, undisturbed negative human urine and synthetic urine exhibited biases of -63% and -46%, respectively. Fully frozen specimens showed a negative bias range of -66% to -76% when allowed to thaw without agitation/mixing prior to analysis when compared to the well-mixed sample from the same container. A single inversion of the source cup/tube yielded a maximum bias of -7.2% when compared to the fully mixed sample.

When those same samples were allowed to sit at room temperature for 3 days, no significant bias was observed (-3.1% to 12% bias in recovery versus fully mixed sample). The depth of aliquoting showed an approximate -20% bias at 2 mm into the liquid, +9.6% at 7 mm into the liquid and a +34% bias when sampled from the bottom of the tube as compared to the fully mixed specimen.

Conclusion/Discussion: Profound biases in recovery of 6-MAM were found in specimens that were frozen and not vigorously mixed prior to analysis.

POSTER

P126: Simplifying Urine Drug Testing with "Flash Hydrolysis" Using New Recombinant beta-Glucuronidase Enzymes

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Background/Introduction: Because of the increased use/misuse of prescription (and illicit) drugs, the prevalence of urine drug testing (UDT) has increased significantly over the past decade and LC-MS/MS is the gold standard for detecting and quantifying drugs in UDT. One ongoing debate in LC-MS/MS UDT is whether to hydrolyze the sample and measure the combined total concentration (glucuronide plus free) or measure glucuronides directly in the analytical method. Each of these protocols has its advantages and disadvantages.

Direct detection of glucuronide conjugates is useful because of the quick and easy sample preparation, therefore reduced consumable and labor costs versus hydrolysis preparations. Furthermore, eliminating the hydrolysis incubation step is more conducive to preparation and analysis as the sample is received instead of batch analysis. However, glucuronide standards can add significant cost, not only due to the price of the standards, but also the overhead of maintaining appropriate QC and documentation for the additional compounds in the test.

Hydrolysis of samples has historically been time consuming, requiring incubation times of up to two hours. Over the past 3-4 years, manufacturers have introduced "second-generation" recombinant β-glucuronidase enzymes that reduce the incubation time to <60 minutes, and even as little as 15 minutes. Although 30-60 minutes may be required for complete hydrolysis of all conjugated metabolites, data show that the majority of the glucuronides are actually hydrolyzed almost immediately when the enzyme is added to the sample. As a result, these new enzymes can simply be added as a "reagent" and the sample analyzed using this "flash hydrolysis" preparation, i.e. without (semi-)lengthy incubation.

Objectives: This presentation will evaluate the advantages/limitations of a novel, quick, room temperature hydrolysis sample preparation using newer β-glucuronidase enzymes when performing LC-MS/MS urine drug testing.

Methods: Drug free urine was fortified with glucuronide standards at concentrations between 2500 ng/mL-20 ug/mL, depending on analyte. Conjugated metabolites represent several drug classes of interest, including opiates/opioids, benzodiazepines, and tricyclic antidepressants. Glucuronides studied were: morphine (morphine-6 and morphine-3), codeine, oxymorphone, lorazepam, oxazepam, temazepam, buprenorphine, norbuprenorphine, naloxone, naltrexone, 6-β-naltrexol, tapentadol, and amitriptyline. A simple dilute-and-shoot sample preparation was utilized according to (modified) manufacturer's recommendations. Two new enzymes were evaluated: B-one Mastermix from Kura Biotec and IMCSzyme RT from IMCS. Samples were analyzed using LC-MS/MS within 2-3 minutes following addition of the β-glucuronidase enzyme. Replicate injections were performed to evaluate continuation of hydrolysis over time from "immediately" up to 60 minutes after addition of enzyme.

Challenging samples from subjects on a prescription medication regimen were also analyzed to evaluate hydrolysis efficiency.

Results: The majority of the conjugates showed complete hydrolysis upon addition of the enzyme and without any incubation except codeine-6-, morphine-6, hydromorphone-, and oxymorphone- glucuronides. Codeine-6- and morphine-6-glucuronides were the most challenging conjugates. Morphine-6-glucuronide was most challenging to hydrolyze and had difficulty reaching 80% completion while stored in a cooled autosampler. Samples from subjects behaved similarly to the fortified samples, with complete hydrolysis of most conjugates upon enzyme addition except for the previously mentioned challenging glucuronides.

Conclusion/Discussion: Results indicate that flash hydrolysis is an efficient sample preparation method for urine drug testing. By using the enzyme as a simple reagent and eliminating an incubation step, most glucuronide conjugates are sufficiently cleaved, reducing the number of compounds that are necessary for direct glucuronide analysis. Overall testing efficiency is improved by reducing the sample preparation time, as well as reducing the number of compounds requiring reference standards and QA/QC (vs direct glucuronide measurement.) Furthermore, the simplified sample preparation eliminates the need for batch preparation of samples, increasing overall efficiency of the laboratory workflow and better accommodation of stat/rush samples.

POSTER

P127: LC-MS/MS Method Development for Legacy and Novel Pharmaceuticals for the Preclinical Screening Platform for Pain (PSPP)

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Background/Introduction: The Preclinical Screening Platform for Pain (PSPP) is a part of the NIH Helping to End Addiction Long-term (HEAL) Initiative. The goal of the initiative is to identify alternative treatments for long-term pain management. These treatments potentially include small molecules, biologics, devices, and natural products. The initial phase of PSPP testing includes the evaluation of collaborator abilities with known compounds which are to be followed by compounds submitted by participating researchers. As a part of the initial phase, time course plasma samples from dosed rats were received from the contract site of the Epilepsy Therapy Screening Program at the University of Utah.

Objectives: The goal of the work was to determine basic pharmacokinetic (PK) parameters for the compounds in rat models.

Methods: A single dose of three compounds; morphine, gabapentin, and celecoxib, were administered separately to male and female rats. The compounds were delivered by intraperitoneal (IP) injection, subcutaneous (SC), or oral administration (PO). Quantitation methods were developed for each of the three compounds of interest using the standard practices of extraction, isolation, and LC-MS/MS analysis. The concentration-time curves obtained were then used to establish preliminary PK information for the compounds.

Results:

		T_{max} (h)	C_{max} (µg/mL)	$t_{1/2}$ (h)	$AUC_{0-\infty}$ (µg*h/mL)
Morphine	Female IP	≤ 0.25	0.460 ± 0.038	0.56 ± 0.02	0.258 ± 0.018
	Male IP	≤ 0.25	0.412 ± 0.054	1.89 ± 0.64	0.403 ± 0.041
	Female SC	≤ 0.25	0.585 ± 0.085	1.75 ± 0.37	0.978 ± 0.118
	Male SC	≤ 0.25	0.639 ± 0.082	1.19 ± 0.03	1.23 ± 0.05
Gabapentin	Female IP	≤ 0.25	82.5 ± 1.6	2.31 ± 0.03	227 ± 13
	Male IP	≤ 0.25	100 ± 12	2.26 ± 0.02	295 ± 3
	Female PO	2	20.3 ± 1.2	2.63 ± 0.01	171 ± 10
	Male PO	2	23.3 ± 0.3	2.83 ± 0.18	285 ± 8
Celecoxib	Female IP	4	1.70 ± 0.16	7.62 ± 1.20	30.8 ± 2.3
	Male IP	4	1.03 ± 0.11	5.22 ± 0.78	16.2 ± 1.2
	Female PO	4	2.51 ± 0.43	10.7 ± 3.7	51.5 ± 9.9
	Male PO	4	1.53 ± 0.31	3.29 ± 0.20	20.1 ± 3.4

AUC values were distinct between routes of administration for all three drugs (p<0.03), while C_{max} was only different for celecoxib (p<0.02). Gabapentin and celecoxib AUC values were distinct between males and females (p<0.001) for both IP and PO administration.

Conclusion/Discussion: Concentrations of morphine, gabapentin, and celecoxib were successfully analyzed from plasma samples collected in rats following IP and PO administration. Differences in AUC and C_{max} were observed between route of administration and sex of the animal. These data support the ability of our center to collect and analyze rat plasma for potential drugs of interest in the PSPP/HEAL initiative study.

POSTER

P128: Optimisation of the Biochip Array DoA Ultra for Enhanced Multiplex Detection of Drugs Related to Impaired Driving

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Background/Introduction: Drug detection involves initial screening of samples for drugs. Drug impaired driving is becoming a major problem worldwide. Biochip array technology allows the simultaneous screening of multiple drugs related to impaired driving from a single undivided sample of blood or urine. By applying the biochip based platform, DoA Ultra Array, amphetamine, barbiturates, benzodiazepines, benzoylecgonine/cocaine, buprenorphine, cannabinoids, dextromethorphan, fentanyl, generic opioids, meprobamate, methadone, methamphetamine, opiates, oxycodone, phencyclidine, tramadol, tricyclic antidepressants and zolpidem can be detected simultaneously, including parent compounds and metabolites. The optimal analytical performance of this array was previously reported (SOFT 2015, 2016).

Objectives: Updated (2017) recommendations for the toxicological investigation of suspected alcohol and drug -impaired driving cases and motor vehicle fatalities have been reported. In view of these recommendations, this study aimed to optimize the DoA Ultra Array for enhanced multiplex detection: a new assay was introduced for the detection of clonazepam, the specificity profile of the assay standardized to oxazepam was expanded to detect oxazepam and temazepam glucuronides and the screening cut-offs of the buprenorphine (blood) and fentanyl (blood and urine) assays were reduced.

Methods: Simultaneous competitive chemiluminescent immunoassays (21), defining discrete test sites on the biochip surface, were employed. The assays were applied to the Evidence series analyzers. The light signal generated from each of the test sites on the biochip was detected using digital imaging technology and compared to that from a stored calibration curve. The signal output is inversely proportional to the concentration of drug in the sample. The systems have dedicated software to process, report and archive the data produced. Sample volume is 10µl (neat urine) and 60µl of 1 in 4 diluted blood.

Results: Results focused on the new and optimized assays are summarized. The addition on the biochip surface of the immunoassay standardized to clonazepam allowed detection of this drug as well as 7 amino clonazepam (cross-reactivity 40%). The assay standardized to oxazepam detected other 17 compounds with cross-reactivity >25%, including oxazepam glucuronide (cross-reactivity: 100.5%), temazepam glucuronide (cross-reactivity: 25.7%). Lorazepam glucuronide presented a cross-reactivity of 15.5%.

The screening cut-off of the new assay standardized to clonazepam was 10ng/mL (blood) 25ng/mL (urine) and the limit of detection (LOD) was <1ng/mL (blood and urine). The cut-off of the assay standardized to oxazepam was 10ng/mL (blood) and 100ng/mL (urine) and the LODs were 5ng/mL and 20ng/mL respectively. The cut-off of the buprenorphine assay (blood) was 1ng/mL with LOD <0.2ng/mL. The cut-off of the fentanyl assay was 1ng/mL for blood and urine with LODs <0.2ng/mL. Overall precision, expressed as CV (%), was <20%.

Conclusion/Discussion: Considering the updated (2017) recommendation for the toxicological investigation of suspected alcohol and drug -impaired driving cases and motor vehicle fatalities, the flexibility of biochip array technology allowed the inclusion of a new assay in the pre-existent DoA Ultra Array for the detection of the Tier 1 compound clonazepam. Moreover, the specificity of one assay standardized to oxazepam was expanded to include detection of glucuronides, which is relevant when screening urine samples. The cut-offs of the buprenorphine (blood) and fentanyl (urine and blood) assays were reduced to 1ng/mL.

In total, with this optimized biochip array >100 compounds can be detected with cross-reactivity >20% including parent compounds and metabolites. This application allows the simultaneous screening of a broad range of drugs related to impaired driving from a single sample. The use of this platform increases the screening capacity in the drug testing process.

Keywords: Biochip array, drugs testing, impaired driving

P129: Biochip Based Multi-Drug Screening of Hair Samples on the Evidence Investigator Analyser

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Background/Introduction: Multi-drug detection facilitates and increases the screening capacity during the drug testing process. The collection of hair samples is non-invasive and the use of this matrix for drug testing, provides a large window of detection as well as the history of drug exposure over time for an individual. Society of Hair Testing (SoHT) and European Workplace Drug Testing Society (EWDTS) guidelines for drug testing in hair are in place.

Objectives: This study reports a new application of biochip array technology to the simultaneous screening of multiple drugs (amphetamine, benzodiazepines, benzoylecgonine/cocaine, cannabinoids, hydrocodone, ketamine, methamphetamine, opiates, oxymorphone and phencyclidine) from a single hair sample on the semi-automated analyser Evidence Investigator.

Methods: Simultaneous competitive chemiluminescent immunoassays, defining discrete test sites on the biochip surface, were employed for the screening of drugs. The immunoassays were applied to the biochip analyser Evidence Investigator. With this system 54 biochips can be handled at a time. The extraction of the drugs from hair samples (50 mg) involved water addition, centrifugation and addition of methanol and zircon pulverising beads. Following spinning and pulverisation, methanol was added. After decantation and drying, the reconstituted sample was added to the biochip. The total assay time including wash, extraction and assay, was 12 hours. Cut-off concentrations, limit of detection (LOD) (17 negative samples were assessed) and inter-assay precision expressed as CV% (n=15 at -50% cut-off, cut-off and +50% cut-off) were determined. Authentic hair samples (n=43) were assessed with the biochip-based technology and LC-MS/MS; the percentage agreement was reported.

Results: The assays presented the following cut-offs and LODs (ng/mg)

Assay	Amphetamine	Benzodiazepines	Benzoylecgonine/Cocaine	Δ9-THC
Cut-off	0.04	0.02	0.2	0.01
LOD	0.032	0.004	0.018	0.008
Assay	THC-COOH	Hydrocodone	Ketamine	Methamphetamine
Cut-off	0.001	0.04	0.5	0.15
LOD	0.0008	0.01	0.354	0.068
Assay	Opiates	Oxymorphone	Phencyclidine	
Cut-off	0.04	0.1	0.02	
LOD	0.02	0.046	0.01	

Inter-assay precision: <18% for all the assays at the concentrations tested. Percentage agreement biochip-based immunoassays and LC-MS/MS (assessment of authentic hair samples): 98% (amphetamine, benzoylecgonine/cocaine), 95% (cannabinoids, opiates) 86% (methamphetamine).

Conclusion/Discussion: Multiple drugs were detected from a single hair sample with the biochip platform. Regarding the cut-offs, for drugs appearing in the SoHT or EWDTS guidelines, the cut-off for ketamine was the same and for amphetamine, benzodiazepines, benzoylecgonine/cocaine, Δ9-THC, methamphetamine and opiates the cut-offs were lower with the biochip platform, reflecting high sensitivity. Favorable agreement with LC-MS/MS was found for the drugs present in the authentic samples. With the Evidence Investigator multiple samples can be assessed at a time (up to 54 biochips can be handled at the same time) which increases even further the screening capacity.

Keywords: Biochip array, drugs testing, hair testing

POSTER

P130: Development of a Novel High-Density Biochip Array for Increased Comprehensive Multi-Drug Screening (44 Simultaneous Assays) from a Single Sample

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Background/Introduction: Biochip array technology enables the detection of multiple drugs from a single sample by incorporating simultaneous immunoassays on the biochip surface, which define discrete test regions (DTRs). Such a multi-analytical approach increases the screening capacity during the drug testing process. With the introduction of new legislation and an increase in drug abuse, the ability to screen as many analytes from a single sample as possible is more important than ever, saving time and resources.

Objectives: In order to increase even further the screening capacity on the biochip surface, this study reports the development of a novel high-density biochip array incorporating 44 test regions for the detection of drugs. This array incorporates immunoassays for the simultaneous detection of acetaminophen, escitalopram, ethylglucuronide, phenylpiperazines as well as 36 other drugs (18 Tier I and 18 Tier II for human blood according to the updated (2017) recommendations for the toxicological investigation of suspected alcohol and drug -impaired driving cases and motor vehicle fatalities. Initial analytical evaluation is presented.

Methods: Simultaneous competitive chemiluminescent immunoassays (n=44) defining DTRs on the biochip surface were employed and applied to the biochip analyser Evidence Investigator. The light signal generated from each of the test regions on the biochip was simultaneously detected using digital imaging technology. The signal output is inversely proportional to the concentration of drug in the sample. The system has dedicated software to process, report and archive the data produced.

Results: The immunoassays on the high-density biochip array and the half maximal inhibitory concentration(IC_{50}) achieved are shown in the table below.

	Immunoassay	IC_{50} (ng/mL)	Tier	Drug Type
1.	6-MAM	0.2	I	Narcotic Analgesics
2.	AB-CHMINACA	0.7	II	Synthetic Cannabinoid
3.	AB-PINACA	0.6	II	Synthetic Cannabinoid
4.	Acetyl fentanyl	0.3	II	Narcotic Analgesics
5.	AH7921	0.1	II	Narcotic Analgesics
6.	alpha PVP	0.2	II	CNS Stimulant
7.	Amphetamine	6.3	I	CNS Stimulant
8.	Barbiturate	5.8	II	CNS Depressant
9.	Mephedrone (bath salts I)	0.4	II	CNS Stimulant
10.	Oxazepam (Benzodiazepine I)	2.4	I	CNS Depressant
11.	Lorazepam (Benzodiazepine II)	1.6	I	CNS Depressant
12.	Buprenorphine	0.2	I	Narcotic Analgesics
13.	Benzoylecgonine/cocaine	1.4	I	CNS Stimulant
14.	Carfentanil	0.1	II	Narcotic Analgesics
15.	Clonazepam	0.1	I	CNS Depressant
16.	Dextromethorphan	0.24	II	Dissociative Drugs
17.	Escitalopram	0.67	-	SSRI CNS Depressant?
18.	Ethylglucuronide	86.2	-	CNS Depressant

19.	Etizolam	0.1	II	CNS Depressant
20.	Fentanyl	0.07	I	Narcotic Analgesics
21.	Fluoxetine	1.9	-	CNS Depressant
22.	Gabapentin	2764	II	CNS Depressant
23.	Ketamine	4.8	II	Dissociative Drugs
24.	LSD	0.1	II	Hallucinogen
25.	Meperidine	0.96	-	Narcotic Analgesics
26.	Meprobamate	10.5	I	CNS Depressant
27.	Methadone	0.4	I	Narcotic Analgesics
28.	Methamphetamine	3.7	I	CNS Stimulant
29.	Mitragynine	0.16	II	CNS Stimulant
30.	Naloxone	0.2	-	Narcotic Analgesics
31.	Opiate	1.9	I	Narcotic Analgesics
32.	Oxycodone I	0.7	I	Narcotic Analgesics
33.	Oxycodone II	0.03	I	Narcotic Analgesics
34.	PCP	0.5	II	Dissociative Drugs
35.	Phenylpiperazines	1.9	-	SARI
36.	Pregabalin	24	II	CNS Depressant
37.	TCA	6.5	II	CNS Depressant
38.	THC	0.9	I	Cannabis
39.	Tramadol	0.2	I	Narcotic Analgesics
40.	Trazodone	0.5	-	CNS Depressant
41.	U47700	0.8	II	Narcotic Analgesics
42.	Generic opioids	1.9	I	Narcotic Analgesics
43.	Acetaminophen	3279.1	-	Analgesic
44.	Zolpidem	0.3	I	CNS Depressant

Conclusion/Discussion: The high-density biochip array developed for the increased comprehensive multi-drug detection (44 simultaneous immunoassays) has the potential of greatly assist the screening step in the drug testing process by increasing even further the test result output from a single sample.

P131: Urine LDTD Shotgun Screening. Drugs of Abuse in Urine at 9 Seconds per Sample Using Dry and Dissolve Easy Preparation

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Background/Introduction: Screening various drug classes in urine samples traditionally require several different immunoassay reagents. In order to reduce the number of screening assays, some laboratories are transitioning to mass spectrometry to allow accurate and broad detection of drugs using a single screening method. The drugs of interest have different polarities and require a long LC chromatographic analysis to separate and elute these different chemical families. To increase the sample analysis throughput, LDTD-MS/MS using fast ß-glucuronide digest and simple extraction method is developed as a screening tool.

Objectives: Using a Dry and Dissolve sample preparation method for LDTD-MS/MS screening of all compounds in a single operation. Screening approach was evaluated for its precision around the cut-off. More than 120 drugs were analyzed in urine from different classes.

Methods: For the Dry and dissolve screening approach, drugs were spiked in urine at a different concentration to evaluate the screening cut-off allowed by this technique. The decision point was determined with the precision test using the 2 SD overlap approach. Individual drugs were spiked in urine, extracted and their potential interferences tested. Positive ion mode was utilized for the following compound groups: benzodiazepine, opiate, amphetamine, antidepressant and more. Other groups were screened using the negative ionization mode: barbiturates and THCC. Urine samples were first hydrolyzed. 10 μL of each urine sample was added in 96 Deep well plates and mixed with 25 μL of the internal standard/Buffer/b-glucuronide (5/20/75) solution. Plate was capped and hydrolysis was performed at 55°C for 30 minutes. After digesting, 400 μL acetonitrile containing 0.5% formic acid was added, mixed and evaporated to dryness. Extract was dissolved with 250 μL MTBE containing 0.05% HCl (1N) followed by 250 μL MTBE containing 0.1% NaOH (1N). 5 μL of sample extract was spotted on a LazWell plate for LDTD-MS/MS screening.

Results: The LDTD-MS/MS source operated in MRM mode allowed for rapid detection of all drugs desorbed simultaneously for screening. Specific transitions were monitored for each drug to quantify calibrator levels.

Spiked samples around the decision point and blank solutions are used to validate the precision of the method. Each concentration must not exceed 20% CV and the mean concentration ± 2 times the standard deviation must not overlap with other concentrations at the decision point. The peak area against the IS ratio was used to normalize the signal. Replicate extractions are deposited on a LazWell plate and dried before analysis. No overlapping at the decision point is observed for all curves and the CV% was below 15% for within-run experiments.

Sample specimens are extracted and analyzed using a LDTD-MS/MS method. After a fast desorption, specimens, fortified and blank samples are evaluated using peak area ratio. All samples having a concentration higher than the cut-off standard are classified as drug positive samples. All samples are analyzed using LC-MS/MS confirmation method. No false positives or false negatives are observed using the LDTD-MS/MS screening method.

Conclusion/Discussion: Luxon Ion Source combined to mass spectrometer system allows ultra-fast, 9 seconds per sample screening of drugs in urine sample using a simple generic sample preparation method. The approach can be set for ''A la carte'' drug list screening.

P132: Rapid Room Temperature Hydrolysis of 16 Glucuronidated Drugs of Abuse using IMCSzyme® RT

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Background/Introduction: β-glucuronidase is an enzyme commonly utilized by clinical and forensic laboratories to remove glucuronic acid conjugated to drug metabolites in biological fluids for improved detection and quantification by mass spectrometry. Several studies indicate the hydrolysis efficiency of β-glucuronidase is highly dependent upon the source of the enzyme and the manufacturing process. A majority of commercially available β-glucuronidases have lower hydrolysis efficiencies against codeine-6-β-glucuronide (C6G), relative to the other glucuronidated analytes, requiring elevated incubation temperatures to achieve accurate recovery of codeine (1-3). We present data to show an enzyme variant that completely hydrolyzes 16 glucuronidated drugs of abuse, including C6G, at room temperature in < 15 minutes. The experiment compares three purified β-glucuronidases working in urine from three different vendors, two synthetic urines and one certified drug free urine.

Objectives: Demonstration of a new β-glucuronidase that rapidly hydrolyzes a recalcitrant substrate, C6G, without high temperature incubation. This reduces the time of hydrolysis and entirely eliminates the need for high temperature incubation equipment from the urine drug testing process, enabling full automation and increased efficiency.

Methods: Two different synthetic urines and a certified drug free urine were fortified with 16 glucuronide standards (Cerilliant) at 5,000 ng/mL each. Conjugates tested represented several different drug classes of interest: anti-depressants (amitriptyline), benzodiazepines (oxazepam, lorazepam, temazepam), opiates (morphine, codeine), opioids (hydromorphone, oxymorphone, dihydrocodeine, 6β-naltrexol, naloxone, tapentadol, O-desmethyltramadol, norbuprenorphine, buprenorphine) and cannabinoids (11-nor-9-Carboxy-Δ9-tetrahydrocannabinol). 50 µL of fortified synthetic urine was mixed with 5 µL of IMCSzyme® RT, 150 µL of vendor-supplied RTB buffer, and 10 µL of internal standard in methanol. Internal standard comprised of all corresponding deuterated substrates of interest. Purified β-glucuronidases from *Brachyspira pilosicoli* (BpGUS) and *Patella vulgata* (PvGUS) were used in the same volume with their optimal hydrolysis buffers. The hydrolyzed urine samples were extracted on Hamilton Microlab NIMBUS using WAX/RP INtip™ chemistry. Each sample was eluted with 100 µL of 1% formic acid in acetonitrile. The eluent was diluted 8-fold with mobile phase A and 5 µL analyzed on a Thermo Scientific™ Vanquish™ UHPLC system coupled with a Thermo Scientific™ Endura™ Triple Quadrupole Mass Spectrometer using a Phenomenex Kinetex® 2.6 µm Phenyl-Hexyl 100 Å, 50 x 4.6 mm LC column. Mobile phase A was 0.1% formic acid in water and mobile phase B was 0.1% formic acid in acetonitrile.

Results: Of all conjugated drug substrates, C6G is the most recalcitrant, with the lowest hydrolysis efficiency by commercially available enzymes. Typically, these enzymes require more than 15 minutes incubation at a temperature at or above 55°C. A next generation β-glucuronidase developed by IMCS—IMCSzyme® RT— hydrolyzes all conjugates in less than 15 minutes, including C6G, at room temperature. Compared to other purified enzymes currently on the market, IMCSzyme® RT achieves 100% C6G hydrolysis; others achieve less than 20%. IMCSzyme® RT results are consistent across all three urines tested. Working at room temperature, IMCSzyme® RT eliminates any need for an incubator or water bath. It also saves time, increasing throughput for high volume clinical and forensic laboratories. IMCSzyme® RT enables complete automation of sample hydrolysis on robotic liquid handling systems, thereby reducing hands-on interventions.

Conclusion/Discussion: IMCSzyme® RT is a new generation of purified β-glucuronidase that allows rapid hydrolysis of all glucuronidated drugs. The hydrolysis efficiency towards C6G is improved by at least 5-fold compared to other commercially available enzymes. The elimination of a heated incubation step provides multiple benefits to increasing throughput and efficiencies in drug testing laboratories.

Keywords: IMCSzyme, β-glucuronidase, instant hydrolysis

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POSTER

P133: Laminar Flow Mass Spectrometry for the Detection and Quantification of Fentanyl, Norfentanyl, and Acetaminophen in Whole Blood

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Background/Introduction: Fentanyl, a synthetic opioid, has been found increasingly in opioid overdose deaths all over the world. With a lethal dose of only 2 mg in most humans, heroin and other drugs laced with fentanyl pose a growing problem on a global scale.

Objectives: This project focused on developing and validating a method for the detection and quantitation of fentanyl, its primary metabolite norfentanyl, and acetaminophen in human whole blood with the use of laminar flow mass spectrometry. It also evaluated the use of Phospholipid Depletion Cartridges (PLD, Biotage, Charlotte, NC, USA) in extracting compounds from the human whole blood (Equitech Enterprises, Kerrville, TX, USA). These compounds were analyzed using UHPLC (PerkinElmer, Waltham, MA, USA) using a Restek Raptor biphenyl 2.7 micron 100 x 3.0 mm (Restek, Bellefonte, PA, USA) with a QSight® 220 CR LC-MS/MS (PerkinElmer, Waltham, MA, USA) in positive ion mode.

Methods: All samples, calibrators, quality controls were prepared by spiking certified reference materials (Cerilliant, Round Rock, TX, USA) into drug-free human whole blood. Calibrators for the calibration model were prepared at 0.5, 1, 5, 10, 25, 50, 100, 250 and 500 ng/ml and QCs concentrations at 1, 75, and 300 ng/mL. Deuterated internal standards of each analyte were spiked into the blood at 200 ng/mL. The limit of detection and quantitation was found to be the lowest calibration point. The LOD was reliably differentiated from the blank matrix by the method developed. The LOQ was detected above the accepted signal-to-noise ratio of ten. Using 100 uL of whole blood and 300 uL of acetonitrile, samples were applied to the PLD cartridges without any conditioning or wash steps then eluted and evaporated under nitrogen. All samples were reconstituted in 90:10 Millipore water (Millipore Milli-Q Ultrapure Type 1 water system, Millipore Sigma, Burlington, MA, USA):Methanol (Fisher Chemical, Fair Lawn, NJ, USA). A binary gradient of 5 mM ammonium formate in Millipore water and methanol, both containing 0.1% formic acid (Sigma-Aldrich, St. Louis, MO, USA), was used for a total run time of 7 minutes. Two ions were monitored for each analyte transition and one for the deuterated standards.

Results: Calibration model, linear dynamic range (LDR), limit of detection (LOD), and limit of quantitation (LOQ) were assessed during method development and validation. All compounds were quantified using a linear model. LOD and LOQ were determined to be 0.5 ng/mL, with LDR of 0.5-500 ng/mL with r^2 values of 0.99 or greater.

Conclusion/Discussion: The method was validated following parameters such as calibration model, linear dynamic range, and limit of detection and quantitation set by ASB Standard 036-Standard Practices for Method Validation in Forensic Toxicology. PLD allowed for an efficient yet effective method for sample extraction for analysis by UHPLC-laminar flow MS/MS, especially when crime laboratories may be overflowing with casework. This method employs a quick and powerful sample preparation technique for a busy laboratory.

POSTER